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(54) **Nucleotide sequences useful as type-specific probes, PCR primers and LCR probes for the amplification and detection of human papilloma virus, and related kits and methods**

Nukleotid-Sequenzen nützlich als typenspezifische Sonden, PCR Primers und LCR Sonden zur Amplifikation und zum Nachweis von humanem Papillomavirus, sowie dazu verwendete Kits und Verfahren

Séquences nucléotidiques utiles comme sondes spécifiques du type amorces de PCR et sondes pour l'amplification et détection du virus-papilloma humain, et kits et procédés utilisés dans ce but

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Description

This invention relates generally to human papilloma virus, and more particularly, relates to nucleotide sequences of short strands of human papilloma virus which can be amplified and/or used to determine the presence of human papilloma virus products in a test sample, and some of which also can be amplified and/or used to determine the specific type of human papilloma virus of types 16 and 18 present in the test sample.

Human papilloma virus (HPV)) is recognized as a venereally-transmitted disease of the anogenital tract which often is associated with the pathogenesis of cervical cancer and its precursor lesions. More than 56 types of HPV have been characterized. Of these, at least 21 types infect the anogenital tract. L. Gregoire et al., J. Clin. Micro 27 (12): 2660- 2665(1989). These mucosotropic viruses are associated most frequently with benign condyloma or latent infections. However, the presence of HPV in premalignant lesions and invasive cancers, particularly of the cervix, may reflect the oncogenic potential of these viruses. See P. M. Howley, in Important Advances in Oncology, D. T. DeVita, Jr. et al., eds., J. B. Lippincott, Philadelphia, PA (1987) at pages 55-73.

Certain HPV types, namely, HPV type 16 and type 18, and to a lesser extent HPV types 31, 33 and 35, are found in a high proportion of invasive cervical cancers and their metastases. However, many HPV types which infect the anogenital tract, such as HPV types 6 and 11, are found most commonly in benign condyloma and only rarely are found in invasive cancers. HPV detected in the anogenital tract can be classified broadly as low risk papilloma viruses (HPV types 6 and 11), intermediate risk papilloma viruses (HPV types 31 , 33 and 35) or high risk papilloma viruses (HPV types 16 and 18), based on the association of the particular HPV type with malignancy. A. T. Lorincz et al., J Nat'l Cancer Inst. 79:671 (1987). Thus, the detection of the presence of HPV and the determination of the specific type of HPV can provide a diagnostic and prognostic tool useful for determining the clinical significance associated with certain HPV types. The early detection of HPV by sensitive and specific reagents and methodologies also could provide earlier therapeutic management and counseling.

A need therefore exists for accurate and reliable methods to identify and type HPV in clinical specimens. However, known polyclonal antisera prepared by immunizing animals with disrupted virions are capable of detecting HPV antigens in only about 30-70% of cutaneous and mucosal warts. Further, the antisera are broadly cross-reactive. Available immunological tests have two major drawbacks. First, only well-differentiated cells apparantly are capable of viral antigen expression. HPV-infected tissues which show higher degrees of neoplasia, such as carcinoma in situ, rarely contain HPV antigen. Thus, the further the development of the malignancy, the smaller the amount of detectable virus in the tested tissue. Secondly, these immunological tests are unable to identify specific viral types.

It is known that papilloma viruses share amino acid sequences in the major capsid proteins. See, for example, C. C. Baker, in The Papovaviridae (Vol. 2), P. M. Howley and N. P. Salzman, eds., Plenum Publ. Corp., New York (1987) at pages 321-385. The DNAs of this virus cross-hybridize, indicating homologous sequences. M. F. Law et al., J. Virol. 58:225-229 (1979). Thus, molecular hybridization techniques have been developed as a more sensitive and specific means of detecting and differentiating HPV DNA and RNA in clinical specimens. See A. T, Lorinez, Obstetrics and Gynecol. Clinics of N. America 14:451 (1987).

Sequences specific for the DNA and RNA of human papilloma virus are known and have been published. See, for example, PCT application No. WO 89/69940 published October 19, 1989, PCT application No. WO 86/05816 published October 9, 1986 and European Patent Application No. 0 301 968 published February 1, 1989.

The molecular hybridization techniques used to detect homologous DNA sequences are sensitive and can be highly specific if used with probes which bind to nucleic acid sequences which are unique to a particular HPV type. However, the concentration of total viral DNA in a given clinical sample may be below the limit of sensitivity of the test. For example, the amount of viral DNA in dysplastic cervical lesions is reduced with increasing dysplasia.

To overcome this problem of sensitivity, viral DNA sequences can be amplified by using, for example, the polymerase chain reaction (PCR) or the ligase chain reaction (LCR) techniques. The products thus obtained can be identified by using conventional hybridization techniques for identification of virus types, such as Southern blotting. See C. Oste, Biotechniques 6:163(1988), K. B. Mullis, U. S. Patent No. 4,683,202, and EP-A-320 308 (BioTechnica).

Both PCR and LCR serve to amplify the DNA present in a test sample to detectable levels. In practice, the level of sensitivity is about 50 to 100 copies per sample. The next most sensitive technique is dot-blot, which can detect about 10,000 molecules, while Southern blot reliably detects about 100,000 copies of DNA per sample.

Thus, the appropriate diagnosis of HPV may require two steps. In one strategy, the presence of a clinically relevant type of HPV is first detected with a group-specific primer. After the presence of HPV is detected, differentiation between types can be performed by using a type-specific probe having low homology between the HPVs of the group. Alternatively, differentiation can be performed using a mixture of type-specific probes at the outset, provided these probes amplify DNA independently of each other, and that they can be detected independently. In the past, such tasks were attempted using specific antibodies. In general, nucleic acid probes and primers allow greater discrimination among subtypes than do antibodies. The use of DNA-based tests increases both sensitivity and specificity over prior-art antibody-based tests.

It therefore would be advantageous to provide oligonucleotide strands of DNA which could be amplified and used to detect the presence, if any, of HPV in a test sample. It also would be advantageous to provide short oligonucleotide strands of DNA which could be amplified and used to detect the presence, if any, of specific types of HPV in the test sample. The combined use of oligonucleotide strands would be advantgeous for allowing for the specific and sensitive in vitro diagnosis of the presence and specific type of HPV present in test samples.

SUMMARY OF THE INVENTION

Oligonucleotides of from about 10 to about 60 nucleotides are provided which can be amplified and used either to detect specific sequences of specific types of human papilloma virus, or consensus regions with high homology among different types. The presence of HPV is determined by contacting the test sample with sequences provided to detect the presence, if any, of HPV types 6, 11, 16, 18, 31, 33 and 61. This may be done with or without prior amplification, for example, by PCR or LCR. Either type-specific or consensus amplification is also possible. Two oligonucleotides are provided if the sequence is to be amplified by PCR, and four oligonucleotides provided if amplification is by LCR, in accordance with these known amplification procedures. After the presence of HPV is detected, the type of HPV present in the sample can be determined by using HPV type-specific probes, by subsequent rounds of PCR, or by LCR. Alternatively, the presence of type-specific HPV can be determined by contacting the test sample directly with type-specific nucleotide sequence provided by the invention for the detection of HPV types 16 and 18. Also provided are methods for using the oligonucleotides and kits for amplifying and detecting the presence of human papilloma virus.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photograph of a gel following electrophoresis showing the results when the primers PCR 1 and PCR5 were used to amplify selected plasmids wherein HPV 6 is in lane 1, HPV 11 is in lane 2, HPV 16 is in lane 3, HPV 18 is in lane 4, and HPV 31 is in lane 5, HPV 33 is in lane 6, HPV 61 is in lane 7, and molecular weight standards are in lane 8.

FIG. 2 is a photograph of a gel following electrophoresis showing the results when the primers PCR 1, PCR2, PCR3, PCR4 and PCR5 were used to amplify plasmid p65.16.8 (HPV 16). PCR1 and PCR5 are primers according to the invention.

FIG. 3 is a photograph of the ethidium bromide-stained gels wherein PCR 1 4 and PCR15 are used in conjunction with IWDO to obtain amplified PCR product.

FIG. 4 is a graph of results obtained from performing LCR on 10^7 molecules of the selected target using LCR5A, LCR5A', LCR5B and LCR5B'. The rate of reaction of 4-methyl lumbelliferone is expressed as fluorescence counts/second/second and plotted against the target HPV type.

FIG. 5 is a graph of results obtained from performing LCR on 10^7 molecules of the selected target using LCR6A, LCR6A', LCR6B and LCR6B'. The rate of reaction of 4-methyllumbelliferone is expressed as fluorescence counts/second/second and plotted against the target HPV type.

FIG. 6 is a graph of results obtained from perfomring LCR on 10^7 molecules of the selected target using LCR7A, LCR7A', LCR7B and LCR7B'. The rate of reaction of 4-methyllumbelliferone is expressed as fluorescence counts/second/second and plotted against the target HPV type.

FIG. 7 is a graph of results obtained from perfomring LCR on 10^7 molecules of the selected target using LCR8A, LCR8A', LCR8B and LCR8B'. The rate of reaction of 4-methyllumbelliferone is expressed as fluorescence counts/second/second and plotted against the target HPV type.

DETAILED DESCRIPTION OF THE INVENTION

The appropriate diagnosis of HPV requires two sets of conditions. The first enables the detection of all pertinent types, and the second set allows differentiation among them. In the past, such tasks have been attempted using specific antibodies. In general, nucleic acid probes and primers allow greater discrimination among subtypes than do antibodies. Thus, the use of DNA-based tests tends to increase both sensitivity and specificity over antibody-based tests.

U. S. Patents No. 4,683,195 and 4,683,202 teach a method of amplifying DNA sequences by using PCR. This method now is a standard procedure in many molecualr biology laboratories. Examples 1-3 which follow below utilize the procedures taught in these two patents and the method as described in the package insert of the commercially-available Gene-Amp™ kit (Document No. 55635-6/89, Perkin-Elmer/Cetus, Emeryville, CA).

In PCR, two complementary polynucleotide strands are amplified by treating the strands with two oligonucleotide primers such that an extension product of each primer is synthesized which is complementary to each nucleic acid strand. The primers are selected such that the extension product of one primer forms a template for the synthesis of an extension product from the other primer once the extension product of the one primer is separated from the template. A chain reaction is maintained by a cycle of denaturing the primer extension products from their templates, treating

the single-stranded molecule generated with the same primers to re-anneal, and allowing the primers to form further extension products. The cycle is repeated for any many times as it takes to increase the target nucleic acid segments to a concentration where they can be detected.

The amplified target sequence can be detected by any of several known techniques; for example, by denaturing the double-stranded products formed by PCR, and treating those products with one or more reporter probes which hybridize with the extension products. The reporter probe has a detectable label, and usually is added in excess. The unhybridized reporter probe, therefore, must be separated from the hybridized reporter probe by involving a separation step. In another method of detecting the extension products without reporter probe and a separation step, the extension products are detected by gels stained with ethidium bromide. The diagnosis can be confirmed by transferring the DNA to nitrocellulose and probing with a probe specific to the HPV type suspected of being present in the sample.

Alternately with PCR, one may take advantage of known restriction sites within the HPV DNA to demonstrate that the amplified DNA contains the expected sequence by examining the cleavage pattern(s) generated with one or more restriction endonucleases. Verifying the authenticity of the amplified sequence may be necessary for two reasons: (1) to ensure that sequences complementary to the amplifying primers are not fortuitously present in cellular DNA which does not contain HPV DNA, and (2), to identify the type of HPV present in the sample. If the sequences chosen for amplification are conserved among HPV types, then the finding of an amplified product does not implicate a particular HPV type. It also should be possible to predict the size of the amplified product based on the binding positions of the two primers. Thus, when that product is found, one reasonably can be assured that HPV is present. However, two different types of HPV may give the same or different size products. Thus, hybridization should be used to confirm the identity of the amplified sequence until confidence is built that the interpretation of the results is reliable. It should be pointed out that the PCR technique will identify only closely related, or type-specific sequences in the absence of highly homologous primers, since only a small portion of the genome is analyzed.

Another particularly useful detection technique is described in EP-A-357 011. In this method, a different reporter molecule, e.g. hapten, is attached to each primer. Following amplification, but before denaturation, duplexes can be detected by "capturing" one hapten (hapten1) with a solid phase coated with anti-hapten1. The separated complex can be detected with a conjugate of label and anti-hapten2, and label associated with the solid phase can be measured.

The Ligase Chain Reaction (LCR) amplifies sections of DNA by copying the section of DNA, and copying the copies of that section of DNA, many times over. This method is described in European Patent Application No. 0 320 308 published June 14, 1989, which is incorporated herein by reference. In this procedure, two probes (for example, A and B) complementary to immediately adjacent regions of a target sequence are hybridized and ligated. This ligated probe then is denatured away from the target, after which it is hybridized with two additional probes (A' and B') of sense opposite to the initial probes A and B. The secondary probes are themselves then ligated. Subsequent cycles of denaturation/hybridization/ligation create the formation of double-length probes of both sense (+) and antisense (-).

In LCR, the nucleic acid of the sample is provided either as single stranded DNA or as double-stranded DNA which is denatured to separate the strands. Four probes are utilized: the first two probes (A and B) are the so-called primary probes, and the second two probes (A' and B') are the so-called secondary probes. The first probe (A) is a single strand capable of hybridizing to a first segment of the primary strand of the target nucleotide sequence. The second probe (b) is capable of hybridizing to a second segment of the primary strand of the target nucleotide sequence. The 5' end of the first segment of the primary strand of the target is positioned relative to the 3' end of the second segment of the primary strand of the target to enable joining of the 3' end of the first probe to the 5' end of the second probe, when the probes are hybridized to the primary strand of the target nucleotide sequence. The third probe (A') is capable of hybridizing to the first probe, and the fourth probe (B') is capable of hybridizing to the second probe (B). The hybridized probes are ligated to form reorganized fused probe sequences. Then, the DNA in the sample is denatured to separate ligated probes from sample DNA. Successive cycles wherein the ligated probes and target DNA undergo the above-described process are performed to increase the amount of detectable DNA in the sample. The amount of cycles performed is dependent upon the sequence used and the sensitivity required of the test. Usually, the cycle can be repeated from 15 to 60 times. At least one of the probes can be conjugated to a signal generating compound.

If the four probes are conjugated to appropriate binding members, the detection of amplified product can be accomplished using standard manual or automated immunoassay procedures known to those skilled in the art. These procedures include, for example, immunochromatography, ELISA, EIA and MEIA. Hybridization also can be accomplished by following standard dot-, slot- or replica-blot procedures which are known to those in the art. The sequences can be labelled with an appropriate signal generating compound (label), which is capable of generating a measureable signal detectable by external means. The various signal generating compounds contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluoroscein and rhodamine, chemiluminescent compounds, radioactive elements such as ^{32}P, and other labels known to those of ordinary skill in the art. The selection of a particular label is not critical, but it will be capable of producing a a signal either by itself or in conjunction with one or more additional substances. A variety of different indicator reagents can be formed of label and specific binding member. Either the label or a specific binding member can be varied. Examples of specific binding members which

can be used as a member of the indicator reagent include antibodies, both monoclonal, polyclonal, and fragments thereof; avidin or biotin, biotin and anti-biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor or an enzyme; an enzyme inhibitor or an enzyme; also any antigenic substances, haptens, antibodies, and combinations thereof.

The test sample can be any biological material suspected of containing HPV. Thus, the test sample can be human body tissue, or a test sample which contains cells suspected of containing HPV.

The invention will now be described by way of Examples, which are meant to describe, but not to limit, the spirit and scope of the invention.

The following terms used in the examples are trademarks, tradenames or chemical abbreviations as specified:

TRIS - chemical abbreviation for [tris(hydroyxmethyl)aminomethane], used as a buffer.
EDTA - chemical abbreviation for ethylenediaminetetraacetic acid, a chelating agent.
FITC - chemical abbreviation for fluorescein isothiocyanate, a flourescent hapten derivative.
NHS-ester - chemical abbreviation for N-hydroxysuccinamide ester
MES - chemical abbreviation for [2-(N-morpholino)ethanesulfonic acid], a buffer.
TWEEN®-20 - trademark of Atlas Chemical for polyoxyethylene sorbitan monolaurate, a detergent.
BIS-TRIS - chemical abbbreviation for [bis-(2-hydroxyethyl)-amino]tris-(hydroxymethyl)methane, a buffer.
TRITON X- 100® - trademark of Rohm & Haas for nonaethylene glycol octylphenol ether, a detergent.
IMx® - trademark of Abbott Laboratories for an automated instrument for performing microparticle enzyme immunoassay (MEIA).

EXAMPLES

EXAMPLE 1

PCR was performed essentially following the package insert of the commercially available Gene-Amp™ kit (document No. 55635-6/89, available from Perkin-Elmer/Cetus, Emeryville, CA). The following reagents were mixed in a 0.5 mL polypropylene tube and used in performing PCR:

Reagent	Final Concentration
Water	(to give final volume = 50 or 100 µL)
Reaction Buffer	10 mM TRIS pH 8.3
	50 mM KC1
	1.5 mM MgC12
	0.01% gelatin
dNTP mixture	200 µM each of dATP,dCTP,dGTP, and TTP
pCR1	1 µM
pCR2	1 µM
plasmid	10 µL 1 ng/100µL
(or control-human placental DNA (Pooled Placental DNA, catalog D-3287, Sigma Chemical Co, St. Louis MO).	
DNA polymerase, Thermus Acquaticus	25 *or* 63.9 units/1 mL

After mixing, the reaction mixture was overlayed with 100 µL of mineral oil. The tube then was placed in an instrument capable of incubation at several temperatures, and subjected to 30 or 40 cycles of programmed temperature change. The precise cycle of temperature change used, and the instrument used, varied with the experiment, and is detailed in the descriptions of the figures in Example 3.

EXAMPLE 2

Following the procedure of Example 1, the following sequences were found to amplify sections of papilloma virus types 6, 11, 16, 18, 31, 33, and 61 using PCR.

PCR1: CAGATGTCTC TGTGGCGGCC TAGTG (ID No. 1)

PCR5: AGGTGTCAGG AAAACCAAAT TTATT (ID No. 5)

PCR14: GAATTAGTTA GACCATTTAA AAG (ID No. 6)

PCR15: GGGGAAACAC CAGAATGGAT A (ID No. 7)

IWDO: ATCATATGCC CACTGTACCA T (ID No. 8)

Sequence IWDO is derived from a sequence disclosed in International application number PCT/US86/00629 (WO 86/05816).

TABLE 1 shows the sequences and where they map to to in the various types.

TABLE 1

SEQUENCES WHICH CAN BE USED AS PROBES OR PCR PRIMERS

PROBE	SEQ ID No.	SEQUENCE	SENSE	MAPS TO: (type 6)	MAPS TO: (type 11)	MAPS TO: (type 16)	MAPS TO: (type 18)	MAPS TO: (type 31)	MAPS TO: (type 33)
PCR1:	1	CAGATGTCTCTGTGGCGGCCTAGTG	+	5786-5810	5768-5792	5634-5658	5610-5634	5550-5574	5591-5615
PCR2:	2	CGTTTTCCATATTTTTTTGCAGATG	+	5767-5791	5749-5773	615-5639	5591-5615	5531-5555	5572-5596
PCR3:	3	AAGTTGTAAGCACCGATGAATATGT	+	5844-5868	5826-5850	695-5719	5671-5695	5611-5635	5652-5676
PCR4:	4	AATGTACCCTAAATACCCTATATTG	-	6008-5984	5990-5966	865-5841	5841-5817	5784-5760	5825-5801
PCR5:	5	AGGTGTCAGGAAAACCAAATTTATT	-	6044-6020	6026-6002	5901-5877	5877-5853	5820-5796	5861-5837
PCR14:	6	GAATTAGTTAGACCATTTAAAAG	+	1495-1517	1495-1517	1524-1546	1595-1617	1462-1484	1518-1540
PCR15:	7	GGGGAAACACCAGAATGGATA	+	1834-1854	1834-1854	1863-1883	1934-1954	1801-1821	1857-1877
IWDO:	8	ATCATATGCCCACTGTACCAT	-	1931-1911	1931-1911	1960-1940	2031-2011	1898-1878	1954-1934

note: PCR2, PCR3 and PCR4 are not probes or PCR primers of the invention

EXAMPLE 3

Linearized plasmids containing full-length papilloma virus inserts in pGEM3 were used as targets. These were pHPV6.1 (HPV6), pSP65.11.5 (HPV 11), p65.16,8 (HPV16), pHPV18H(HPV18), pG3 HPV31 (HPV31), pLNK322.HPV33 (HPV33), and pBR322.HPV61 (HPV61). The Programmable Cyclic Reactor™ (available from Ericomp, San Diego) was used as the incubation instrument. Following PCR procedures as described in Example 1,10 µL aliquots were analyzed by electrophoresis through agarose (comprising a 5:3 ratio of NuSieve®:SeaKem® GTG, available from the FMC Corp., Rockland, ME) in a buffer comprising 0.089 M TRIS, 0.089 M borate, 2 mM EDTA, and 0.5 ppt ethidium bromide.

FIG. 1 is a photograph of an ethidium bromide-stained 1.2% agarose gel showing results using 63.9 units/mL DNA polymerase, in the DNA Thermal Cycler™ (Perkin-Elmer/CETUS, Emeryville, CA). The samples were heated for 5 minutes at 94°C, then subjected to 40 cycles of a temperature program of: 1 minute at 94°C, 2 minutes at 40°C, and 1.5 minutes at 72°C. The PCR primers used in this case were PCR 1 and PCR5 of Example 2. Examination of the gel following electrophoresis showed bands at the expected positions, i.e. 292 bp. Lane 1, HPV6; lane 2, HPV 11; lane 3, HPV16 ; lane 4, HPV 18; lane 5, HPV31; lane 6, HPV33, lane 7, HPV61; lane 8, pooled human placental DNA (suspected of having HPV infection); lane 9, molecular weight markers-Hae III digest of ΦX174.

FIG. 2 is a photograph of an ethidium bromide-stained 4% agarose gel showing results using 25 units/mL DNA polymerase, in the Programmable Cycler Reactor™ (Ericomp, San Diego, CA). Samples in this case were subjected to 30 cycles of a temperature program of: 50°C for one (1) minute, 72°C for two (2) minutes and 95°C for one (1)) minute. In this case, the primers PCR1 , PCR2, PCR3, PCR4 and PCR5 of Example 2 were used to amplify plasmid

p65,16,8(HPV 16). Examination of the gel of Figure 2 shows bands at the expected positions, i.e., PCR 1 and PCR4, 235 bp, lane 2; PCR1 and PCR5, 267 bp, lane 4; PCR2 and PCR4, 254 bp, lane 6; PCR2 and PCR5, 286 bp, lane 8; PCR3 and PCR4, 174 bp, lane 10; PCR3 and PCR5, 206 bp, lane 12; molecular weight marker, 123, 246, 369, 492,... bp ladder, lane 1. Note footnote to Table 1.

FIG. 3 is a photograph of an ethidium bromide-stained 1.2% agarose gel showing results using the same conditions as FIG. 1. In this case, PCR14 and PCR15 were used as primers in conjunction with IWDO. The expected size of the amplified PCR product of PCR 14 and IWDO is 437 bp for all of the HPV types tested. The expected size of the product of PCR 15 and IWDO is 98 bp. Products of these sizes appear in the gels, confirming that PCR14 and PCR15, used in conjunction with IWDO, will amplify HPV DNA of types 6, 11, 16, 18, 31 , 33, and 61. Lane 1, Molecular weight marker (Hae III digest of FX 174); PCR 14 + IWDO, lanes 2-9: lane 2, HPV6 ; lane 3, HPV 11 ; lane 4, HPV16 ; lane 5, HPV18 ; lane 6, HPV31; lane 7, HPV33; lane 8, HPV61 ; lane 9, human placental DNA suspected of being infected with HPV; PCR 5 + IWDO, lanes 10-17: lane 10, HPV6 ; lone 11 , HPV 11; lane 12, HPV16; lane 13, HPV18 ; lone 14, HPV31 ; lane 15, HPV33 ; lane 16, HPV61 ; lane 17, human placental DNA suspected of being infected with HPV; lane 18, molecular weight marker (Hae III digest of FX174 and HinD III digest of 1 DNA).

EXAMPLE 4

The following reagents were mixed in a 0.5 mL polypropylene tube as follows for the Ligase Chain Reaction (LCR):

Reagent	Volume	Final Concentration
Water	21 µL	
Reaction Buffer	10 µL	50 mM EPPS pH7.8 10 mM NH_4Cl 10 mM $MgCl_2$ 100 mM K^+ (from all sources) 0.001% BSA 1 mM DDT
Nicotine Adenine Dinucleotide (NAD)	0.5 µL	100 µL
ProbeA (sense)	4 µL	5.0×10^{11} molecules
ProbeA' (antisense, 5'-phosphate)	4 µL	7.5×10^{11} molecules
ProbeB (sense, 5'-phosphate)	4 µL	7.5×10^{11} molecules
Probe B' (antisense)	4 µL	5.0×10^{11} molecules
Target (including human placental carrier DNA at 10 µg/mL)	1.5 µL	15 ng/50 µL
DNA ligase, Thermus therpophilus	1 µL	

This reaction mixture was overlayed with 30 µL of mineral oil. The tube was placed in an instrument capable of incubation at several temperatures (e.g. thermal cycler from Coy Laboratory Products (Ann Arbor, MI) or the Programmable Cycler Reactor™ (available from Ericomp, San Diego, CA), and then subjected to several cycles of programmed temperature change. Each cycle involved incubation at 50°C for one minute and 85°C for one minute.

EXAMPLE 5

The following procedure was used when performing the Ligase Chain Reaction (LCR), which is described in published European Patent Application No. 0 320 308 A2. The reagents of Example 4 were utilized in the procedure as follows: Two probes (A and B) complementary to immediately adjacent to regions of a target sequence were hybridized and ligated. This ligated probe was denatured away from the target, and hybridized with two additional probes (A' and B') of sense opposite to the initial probes (A and B). The secondary probes then were ligated. Subsequent cycles of denaturation/hybridization/ligation created the formation of double-length probes of both + and - sense.

EXAMPLE 8

The following sequences were determined to be specific for a portion of the E6 region of HPV type 16:

Probe	SEQ ID No.	Sequence	Maps to:
LCR5A	81	GCTGCAAACA ACTATACATG ATATAA	157 - 182
LCR5A'	82	pTTATATCATG TATAGTTGTT TGCAGC	182 - 157
LCR5B	83	pTATTAGAATG TGTGTACTGC AAGCA	183 - 208
LCR5B'	84	TGCTTGCAGT ACACACATTC TAATA	208 - 157

EXAMPLE 9

Base-denatured plasmids which contained full-length papilloma virus inserts in pGEM3 were used as targets. These plasm ids were pG3HPV6(+) (HPV6), pSP 65. 11.5 (HPV11), pSP65.168 (HPV16), p63HPV18H(-)(HPV18), p63:HPV31 (HPV31), pLNK322:HPV33 (HPV33), pBR322:HPV35 (HPV35), pUC19:HPV52 (HPV52), pLNK322: HPV58 (HPV58), pUC9:HPV59 (HPV59) and PBR322:HPV61 (HPV61). All of the oligonucleotides used as probes from Example 8 had chemical labels covalently attched at the ends distal from ligation. These labels were: 5'-fluorescein-LCRSA, 3'-fluorescein-LCRSA', 3'- biotin-LCR5B and 5'-biotin-LCR5B'. Covalent attachment was performed by known methods, i.e., reaction of amine-terminated oligonucleotides with FITC or biotin-NHS-ester essentially following the procedures of Kansal et al., Tet. Letters 29:5537-5540 (1988). The thermal cycler used was obtained from Coy Laboratory Products, Ann Arbor, MI.

Following the LCR procedure of Examples 4 and 5, the mixtures were analyzed using a prototype version of the IM_x® instrument (Abbott Laboratories, Abbott Park, IL), following the protocol for microparticle enzyme immunoassays as follows. A 40µL aliquot of an LCR mixture was diluted 1:1 with distilled water. This diluted mixture was incubated with 50µL antifluorescein-conjugated polystyrene microparticles for five (5) minutes to form a suspension of immune complexes on the microparticles. This suspension then was transferred to an inert glass fiber matrix, to which the microparticles became attached. The matrix was washed with buffer (0.3M Nacl, 10 mM TRIS pH8, 0,1%NaN_3), Any immune complexes attached to the glass matrix was detected by using alkaline phosphatase-labeled conjugate that catalyzed the hydrolysis of 4-methylumbelliferone. The rate at which the 4-methylumbelliferone was generated on the matrix was proportional to the concentration of LCR product formed in the reaction mixture.

Referring to FIG. 4, the graph shows the results obtained from performing LCR on 10^7 molecules of the targets in shown. The rate shown is the rate of generation of 4-methylumbelliferone, and is expresssed as fluorescence counts/second/second. Background signal is approximately 10 c/s/s, as shown by the amplification of human placental DNA. The only values above background are those for sample containing HPV16, and those values are about 60 times background signal.

EXAMPLE 10

The following sequences were determined to be specific for a portion of the E6 region of HPV type 18:

Probe	SEQ ID No.	Sequence	Maps to:
LCR6A	85	CTTCACTGCA AGACATACAA ATAA	172 - 195
LCR6A'	86	pTTATTTCTAT GTCTTGCAGT GAA	195 - 173
LCR6B	87	pCCTGTGTATA TTGCAAGACA GTAT	196 - 219
LCR6B'	88	TACTGTCTTG CAATATACAC AGG	218 - 196

EXAMPLE 11

Plasmids which contained full-length papilloma virus inserts in pGEM3 were used as targets. The plasmids used were those described in Example 9 . All of the oligonucleotides used as probes obtained from Example 10 had chemical labels covalently attached at the ends distal from ligation. The thermal cycler was obtained from Coy Laboratory Products, Ann Arbor, MI.

Following LCR procedure described in Examples 4 and 5, the mixtures were analyzed as described in Example 9 using the prototype version of the IM_x® instrument (Abbott Laboratories, Abbott Park, IL).

Referring to FIG. 5, the graph dislays the results obtained from performing LCR on 10^7 molecules of the targets. The rate shown is the rate of generation of 4-methylumbelliferone, and is expressed as fluorescence counts/second/

second. Background signal is approximately 15 c/s/s, as shown by the amplification of human placental DNA. The only values above background are those for sample containing HPV 8, and those values are about 40 times background signal.

EXAMPLE 12

The following sequences were determined to be specific for a portion of the E6 region of HPV type 18:

Probe	SEQ ID No.	Sequence	Maps to:
LCR7A	89	TATATTGCAA GACAGTATTG GAAC	200 - 223
LCR7A'	90	pGTTCCAATAC TGTCTTGCAA TTTA	223 - 200
LCR7B	91	pTTACAGAGGT ATTTGAATTT GCATT	224 - 249
LCR7B'	92	AATGCAAATT CAAATACCTC TGTAA	249 - 224

EXAMPLE 13

Plasmids which contained full-length papilloma virus inserts in pGEM3 were used as targets. The plasmids were those of Example 9 All of the oligonucleotides from Example 12 which were used as probes had chemical labels covalently attached at the ends distal from ligation. The thermal cycler was as described in Example 11.

Following the LCR procedure of Examples 4 and 5, the mixtures were analyzed as described in Example 9 using the prototype version of the IMx instrument (Abbott Laboratories, Abbott Park, IL).

Referring to FIG. 6 , the graph shows the results obtained from performing LCR on 10^7 molecules of the targets. The rate shown is the rate of generation of 4-methylumbelliferone, and is expressed as fluorescence counts/second/second. Background signal is approximately 15 c/s/s, as shown by the amplification of human placental DNA. The only values above background are those for sample containing HPV 18, and those values are about 80 times background signal.

EXAMPLE 14

The following sequences were determined to be specific for a portion of the E6 region of HPV type 16.

Probe	SEQ ID No.	Sequence	Maps to:
LCR8A	93	GTATGGAACA ACATTAGAAC AGCA	352 - 375
LCR8A'	94	pTGCTGTTCTA ATGTTGTTCC ATAC	375 - 352
LCR8B	95	pATACAACAAA CCGTTGTGTG ATTT	376 - 399
LCR8B'	96	AAATCACACA ACGGTTTGTT GTAT	399 - 376

EXAMPLE 15

Plasmids which contained full-length papilloma virus inserts in pGEM3 were used as targets. All of the oligonucleotides from Example 14 used as probes had chemical labels covalently attached at the ends distal from ligation. The thermal cycler was as described in Example 11.

Following LCR procedure of Examples 4 and 5, the mixtureswere analyzed as described in Example 9 using the prototype version of the IM_x® instrument (Abbott Laboratories, Abbott Park, IL).

Referring to FIG. 7 , the graph details the results obtained from performing LCR on 10^7 molecules of the targets. The rate shown is the rate of generation of 4-methylumbelliferone, and is expressed as fluorescence counts/second/second. Background signal is approximately 10 c/s/s, as shown by the amplification of human placental DNA. The only values above background are those for sample containing HPV 16, and those values are about 36 times background signal.

EXAMPLE 16

The attached Appendix (example 16) discloses the sequences of the invention aligned to known sequences.

EXAMPLE 16

APPENDIX

HUMAN PAPILLOMA VIRUS

ALIGNMENT of TYPES 6, 11, 16, 18, 31, and 33; with CONSENSUS SEQUENCE

The appendix lists the sequences of HPV types 6, 11, 16, 18, 31, and 33. It also shows where the sequences of this invention line up with respect to these HPV sequences. In addition, the appendix shows where other sequences, known to the Inventors as of 28 September 1990, and claimed or disclosed by or unknown to others, line up with respect to these sequences.

1. Sequences and Regions Claimed by Us;

PCR = Sequences per examples 1 through 3 (only PCR1, PCR5 PCR14 and PCR15)

LCR = Sequences per examples 4 through 14 only

2. Sequences and Regions Unknown to Others and Not Claimed by Us;

PCR = Sequences designated PCR other than those above JJ

LCR = Sequences designated LCR other than those above

3. *Sequences and Regions Claimed by Others;*
(Italics represents antisense sequences)

AUS = International application number (Australians) PCT/AU88/00047 (WO 88/06634)

WL = International application number (Wayne Lancaster, Wayne State University) PCT/US86/00629 (WO 86/05816)

BE = European Patent Application (Belgians) 89.033834 (X= T or U)

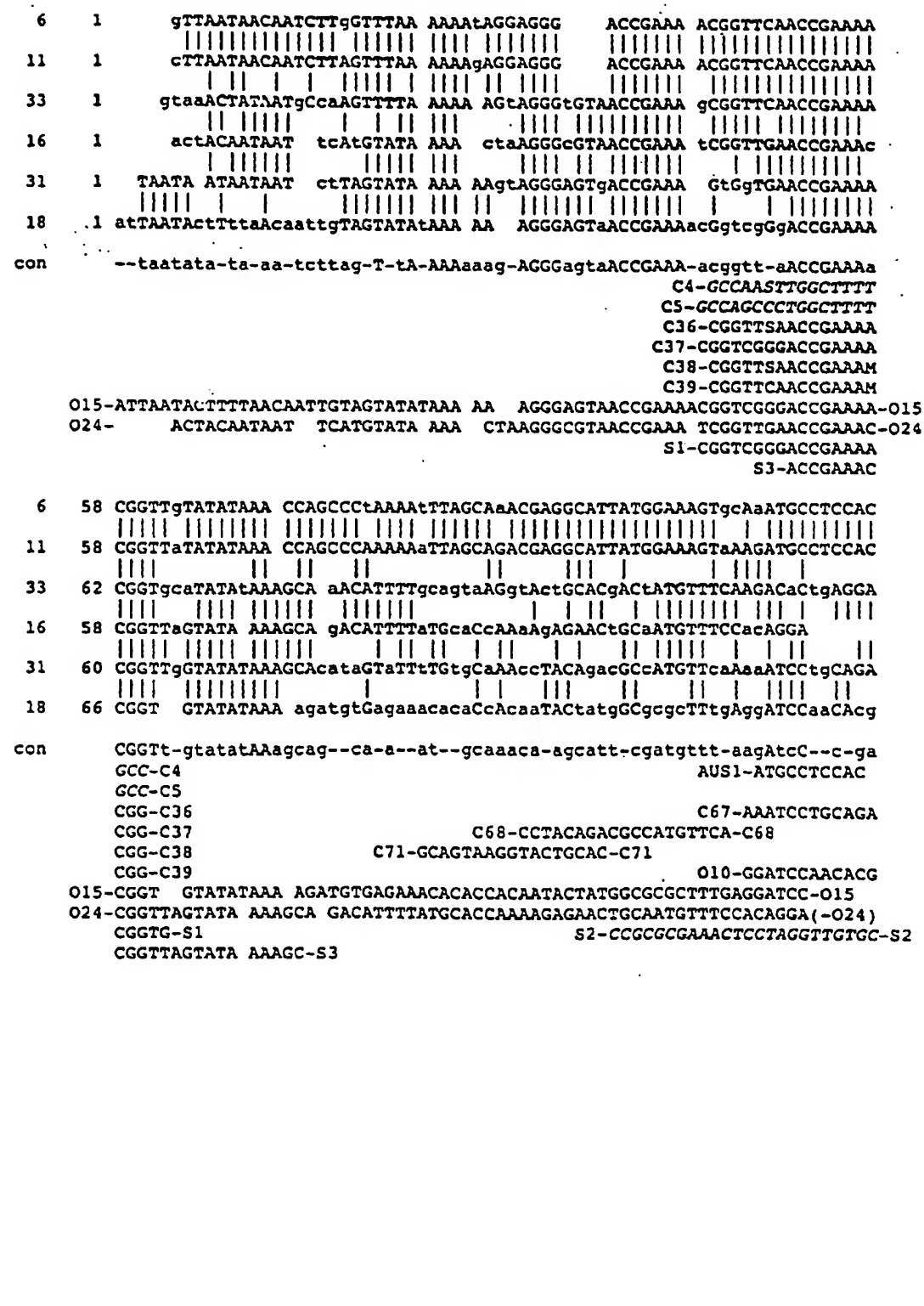
C = International application number (CETUS) PCT/US89/03747 (WO 90/C2821)

O = International application number (Oncor) PCT/US89/O1318 (WO 89/09940)

and

4. Sequences and Regions Disclosed by Others.

S = Sarkar, F.H. and Crissman. J.D. *Biotechniques* **9** 180-184 (1990) *(Italics represents antisense sequences)*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11 1 cTTAATAACAATCTTAGTTTAA AAAAgAGGAGGG ACCGAAA ACGGTTCAACCGAAAA
33 1 gtaaACTATAATgCcaAGTTTTA AAAA AGtAGGGtGTAACCGAAA gCGGTTCAACCGAAAA
16 1 actACAATAAT tcAtGTATA AAA ctaAGGGcGTAACCGAAA tCGGTTGAACCGAAAc
31 1 TAATA ATAATAAT ctTAGTATA AAA AAgtAGGGAGTgACCGAAA GtGgTGAACCGAAAA
18 1 atTAATActTttaAcaattgTAGTATAtAAA AA AGGGAGTaACCGAAAacGgtcgGgACCGAAAA
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C5-GCCAGCCCTGGCTTTT
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C37-CGGTCGGGACCGAAAA
C38-CGGTTSAACCGAAAM
C39-CGGTTCAACCGAAAM
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S3-ACCGAAAC
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11 58 CGGTTaTATATAAA CCAGCCCAAAAAaTTAGCAGACGAGGCATTATGGAAAGTaAAGATGCCTCCAC
33 62 CGGTgcaTATAtAAAGCA aACATTTTgcagtaAGgtActGCACgACtATGTTTCAAGACaCtgAGGA
16 58 CGGTTaGTATA AAAGCA gACATTTTaTGcaCcAAaAgAGAACtGCaATGTTTCCacAGGA
31 60 CGGTTgGTATATAAAGCAcataGTaTTtTGtgCaAAccTACAgacGCcATGTTcaAaaATCCtgCAGA
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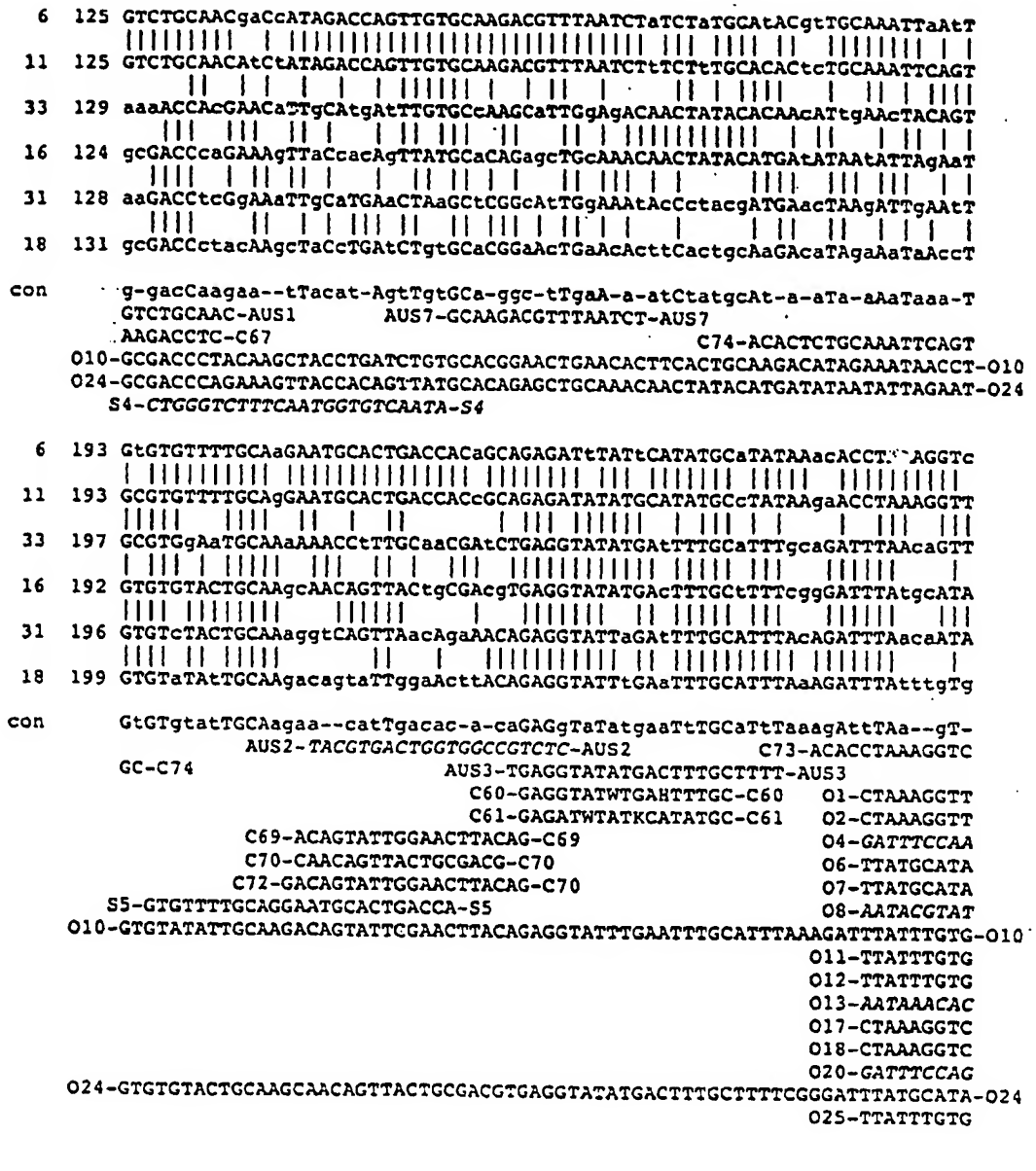
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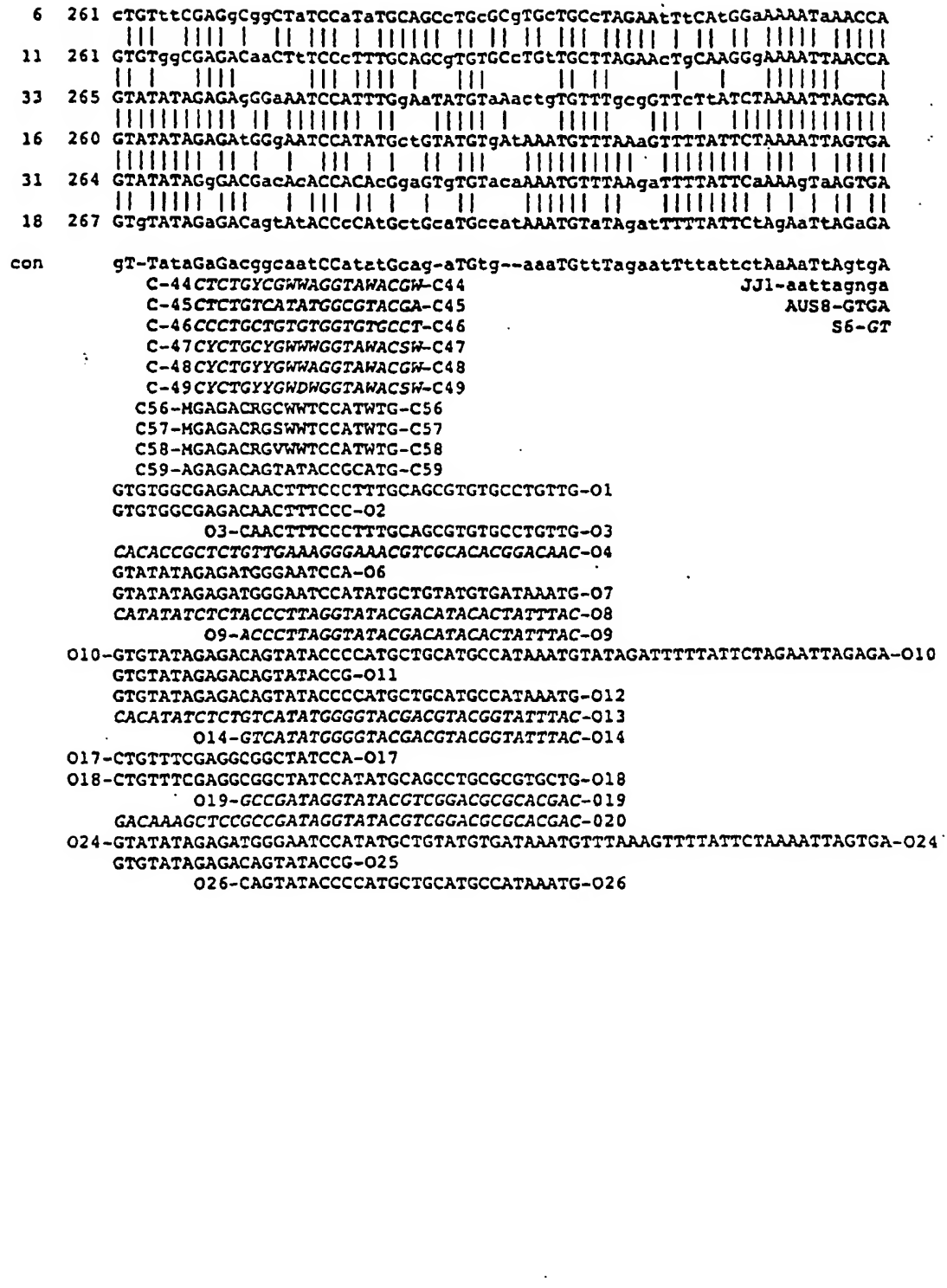
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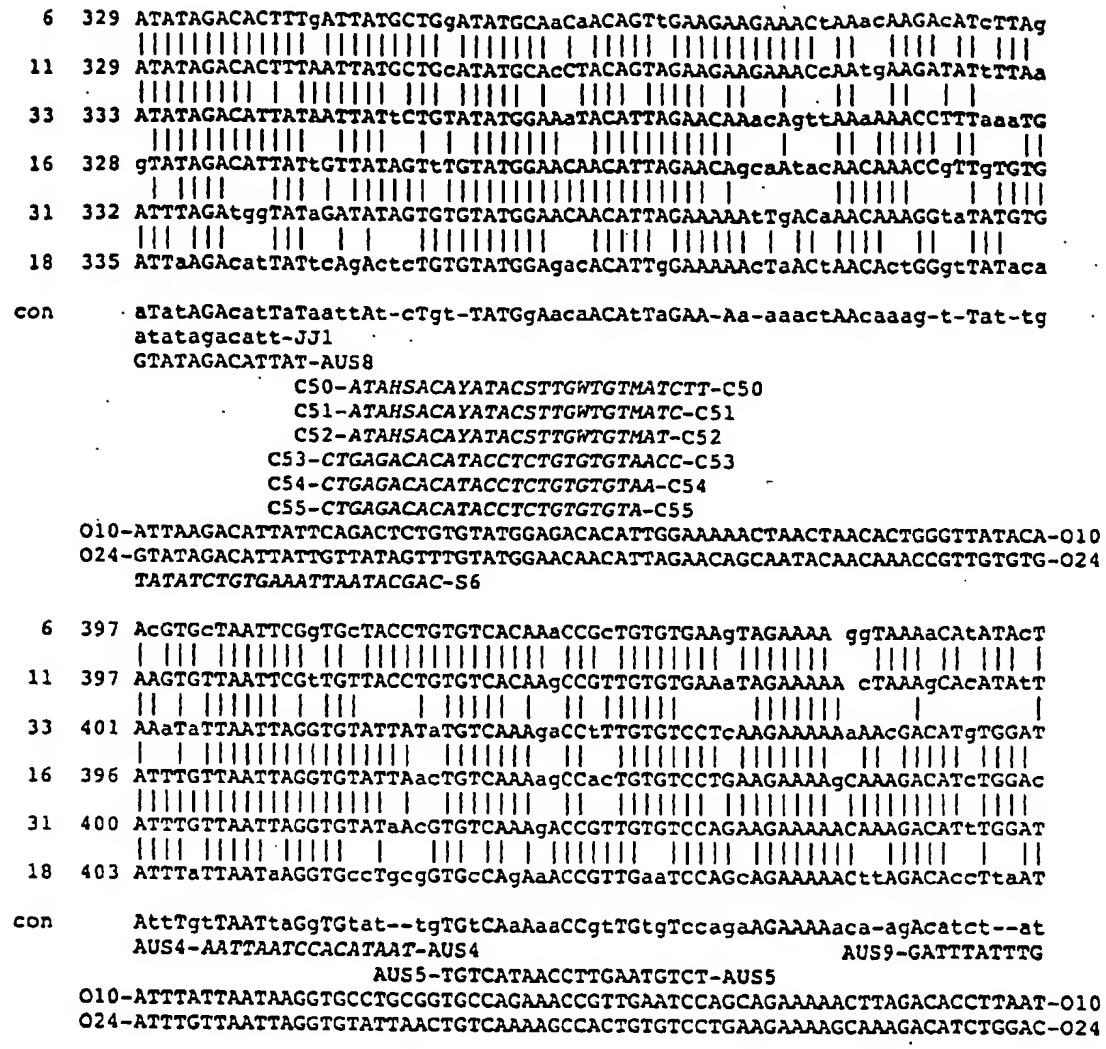
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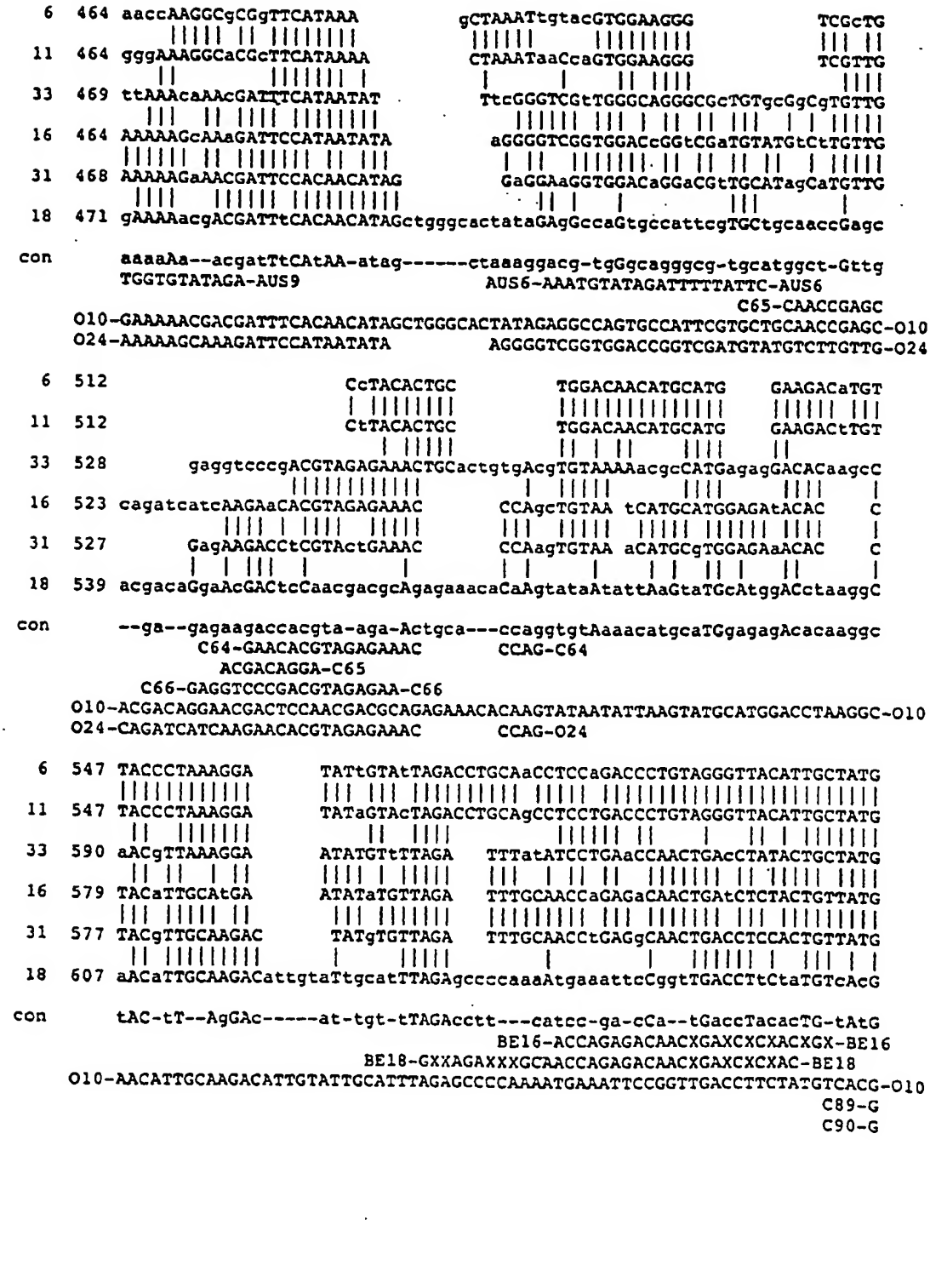

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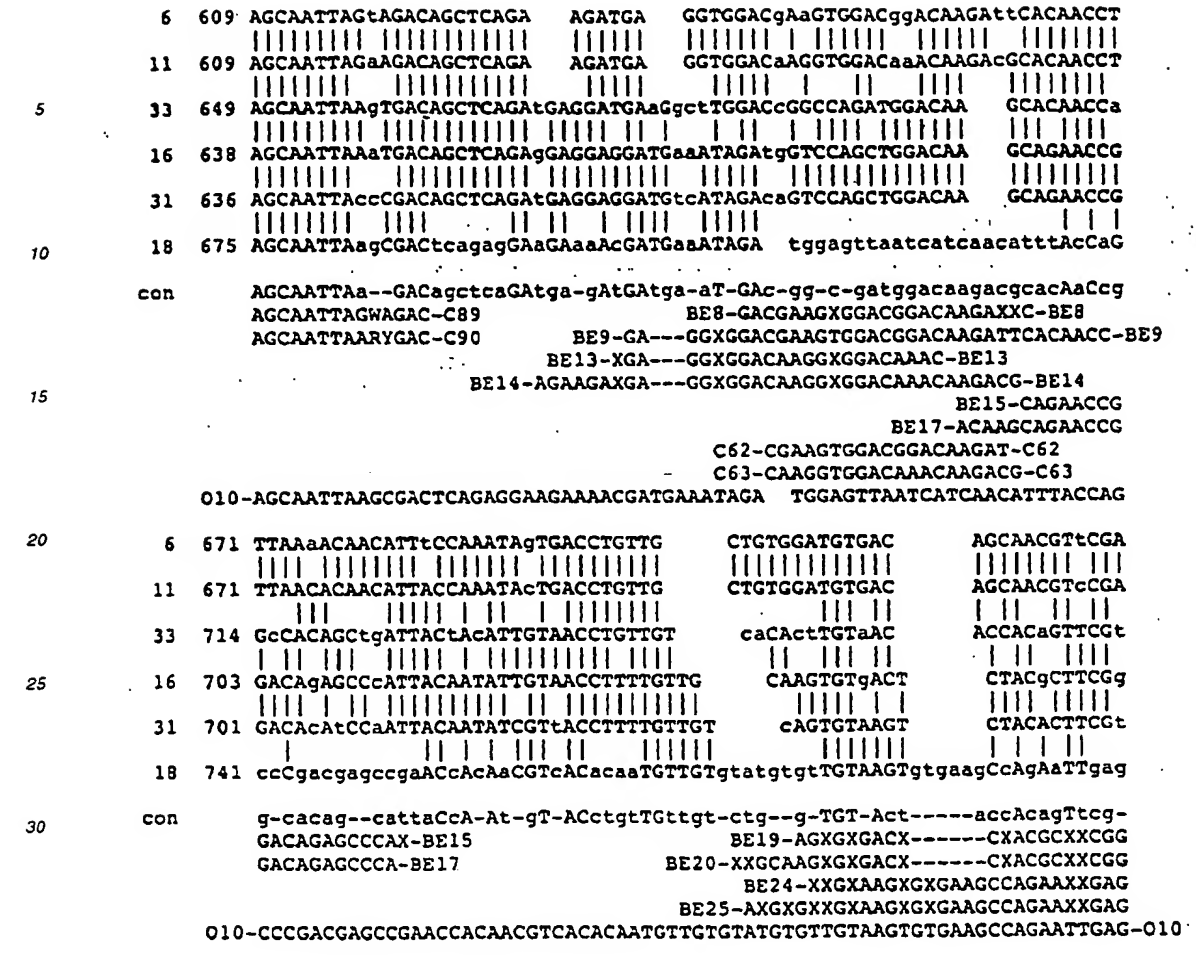
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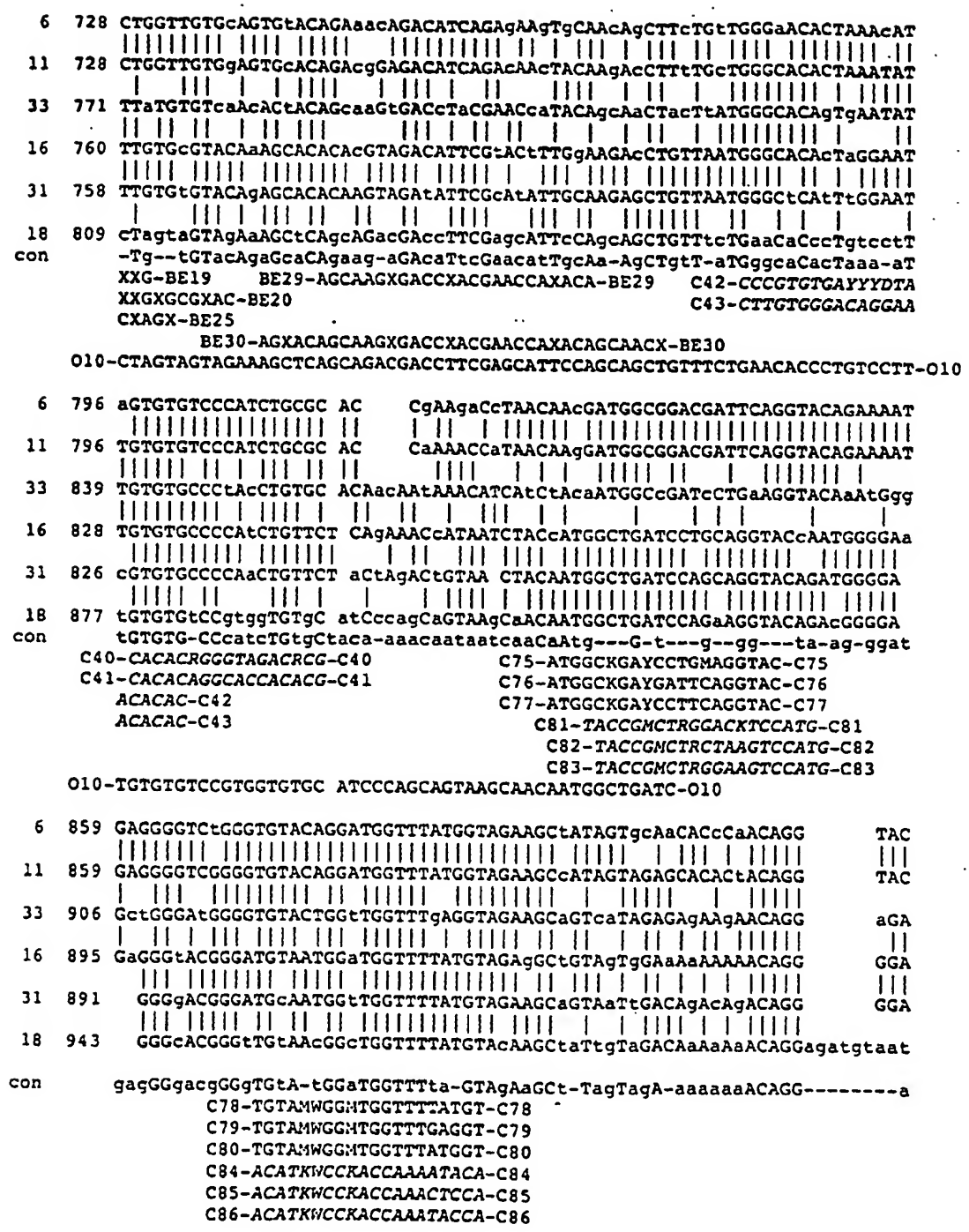
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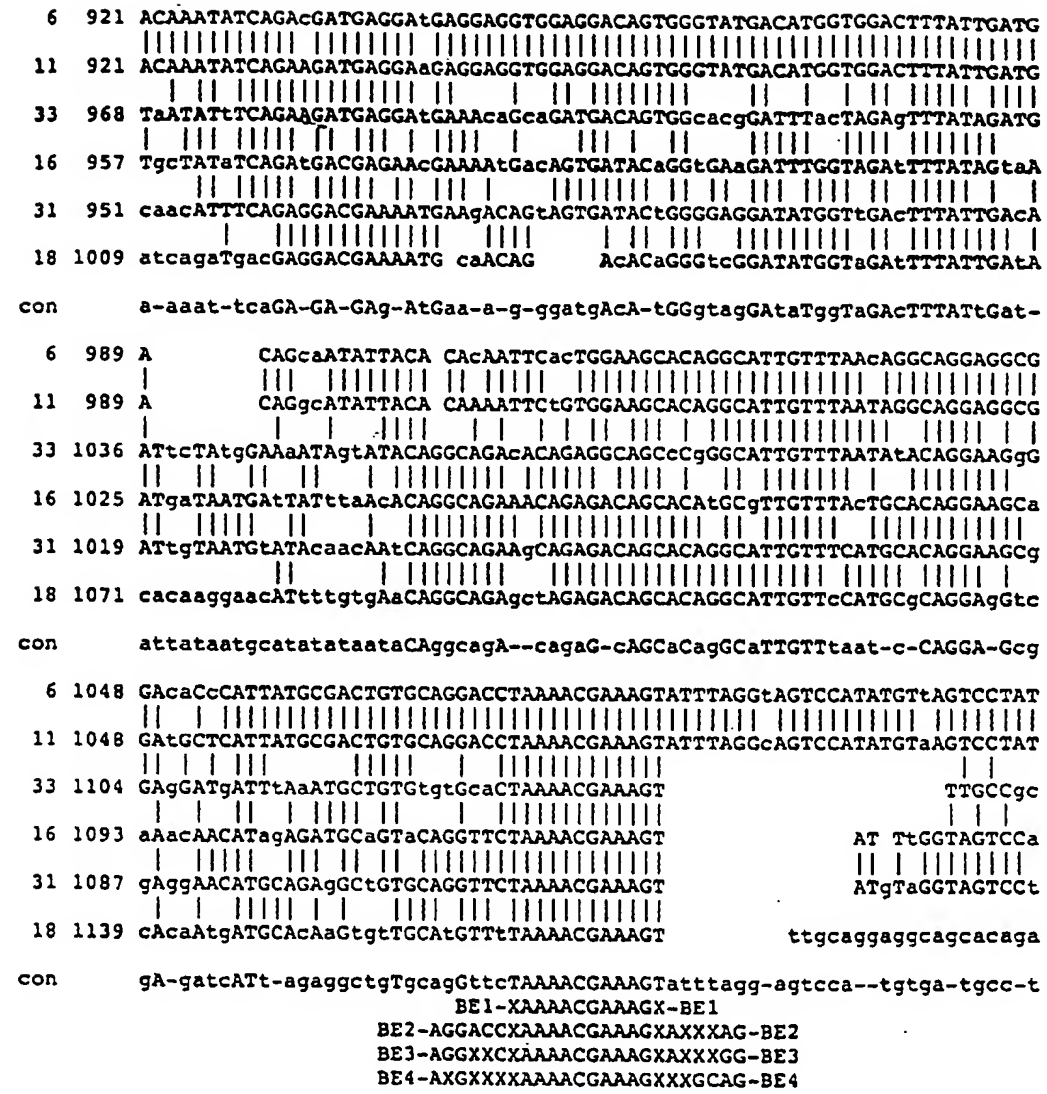
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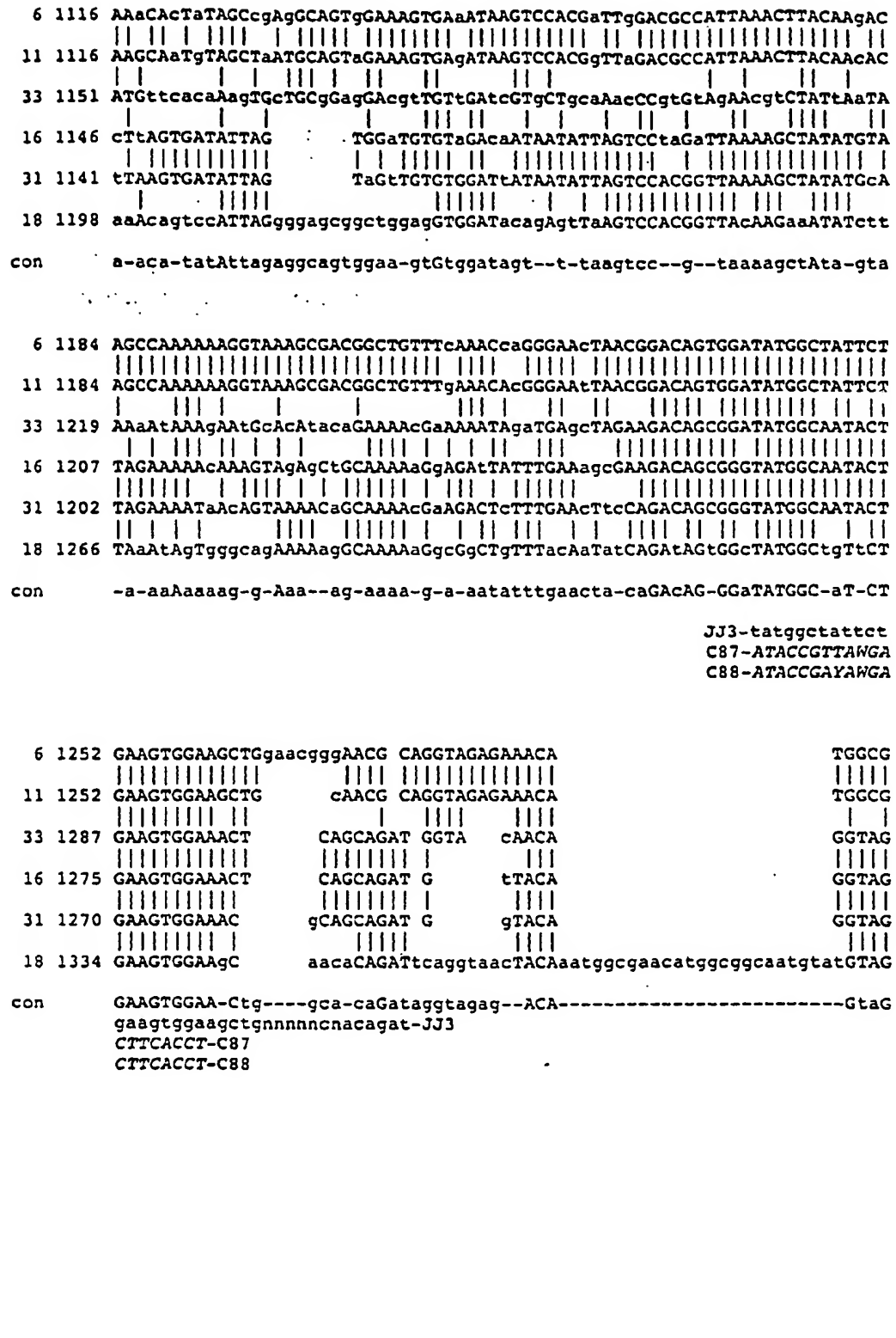
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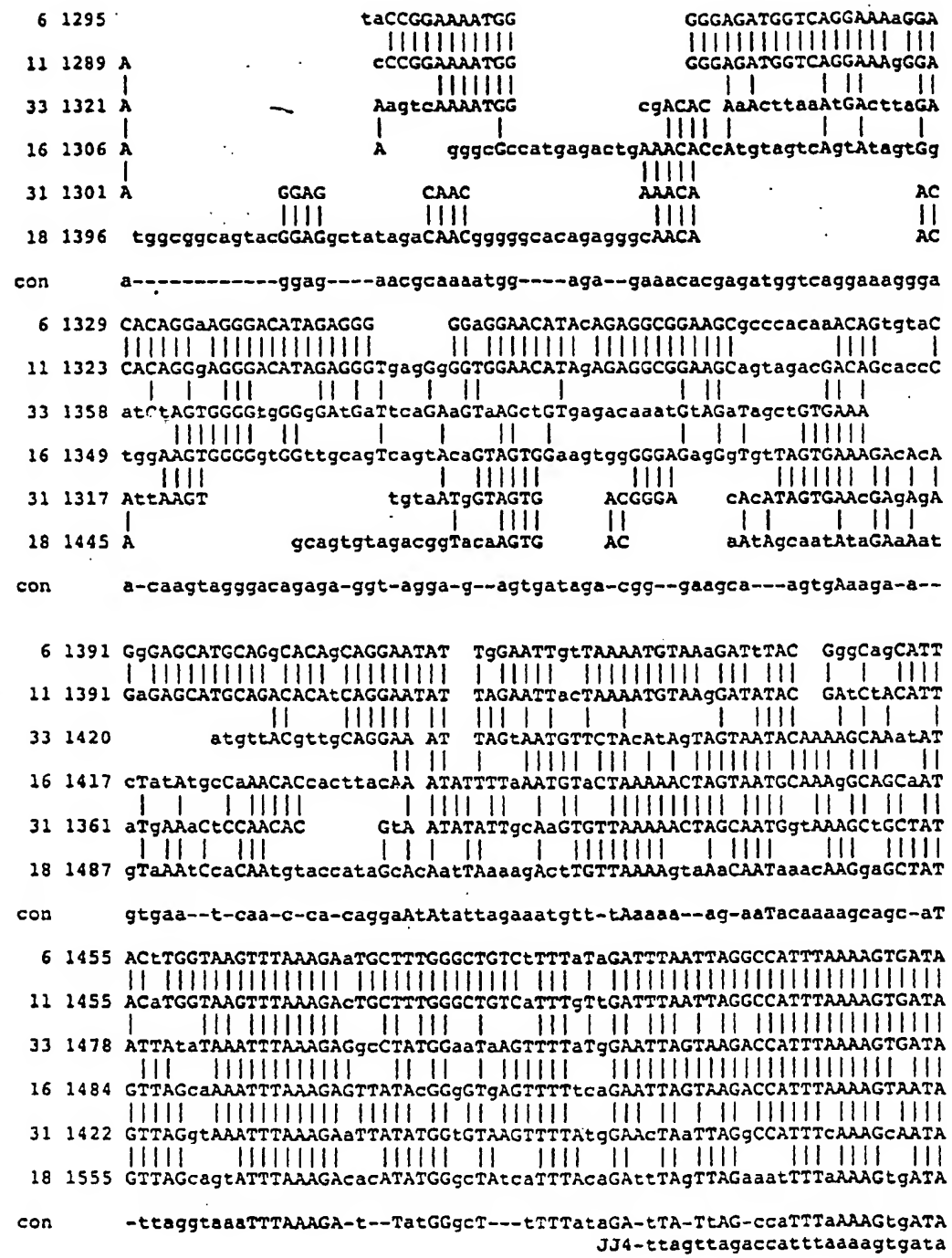

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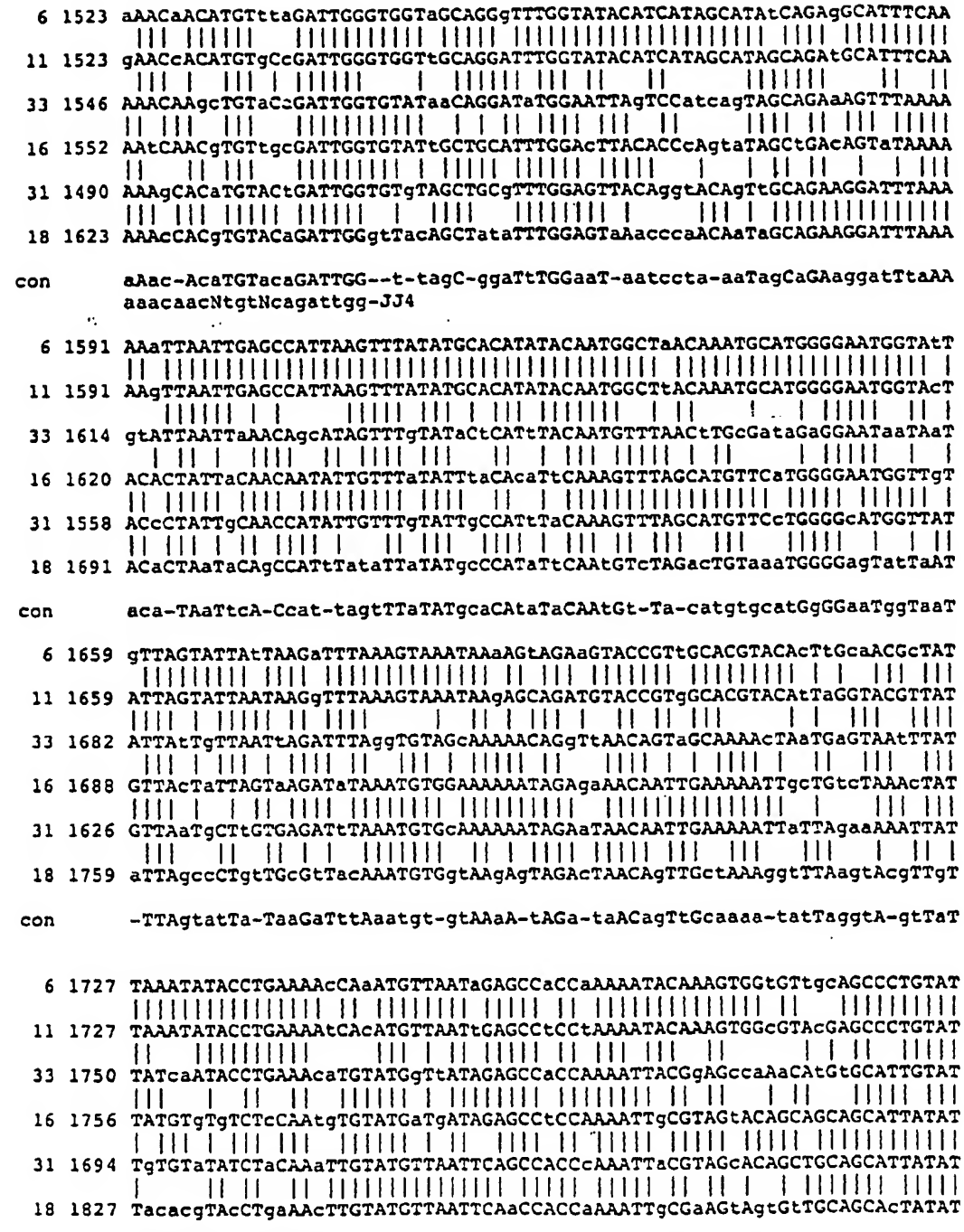
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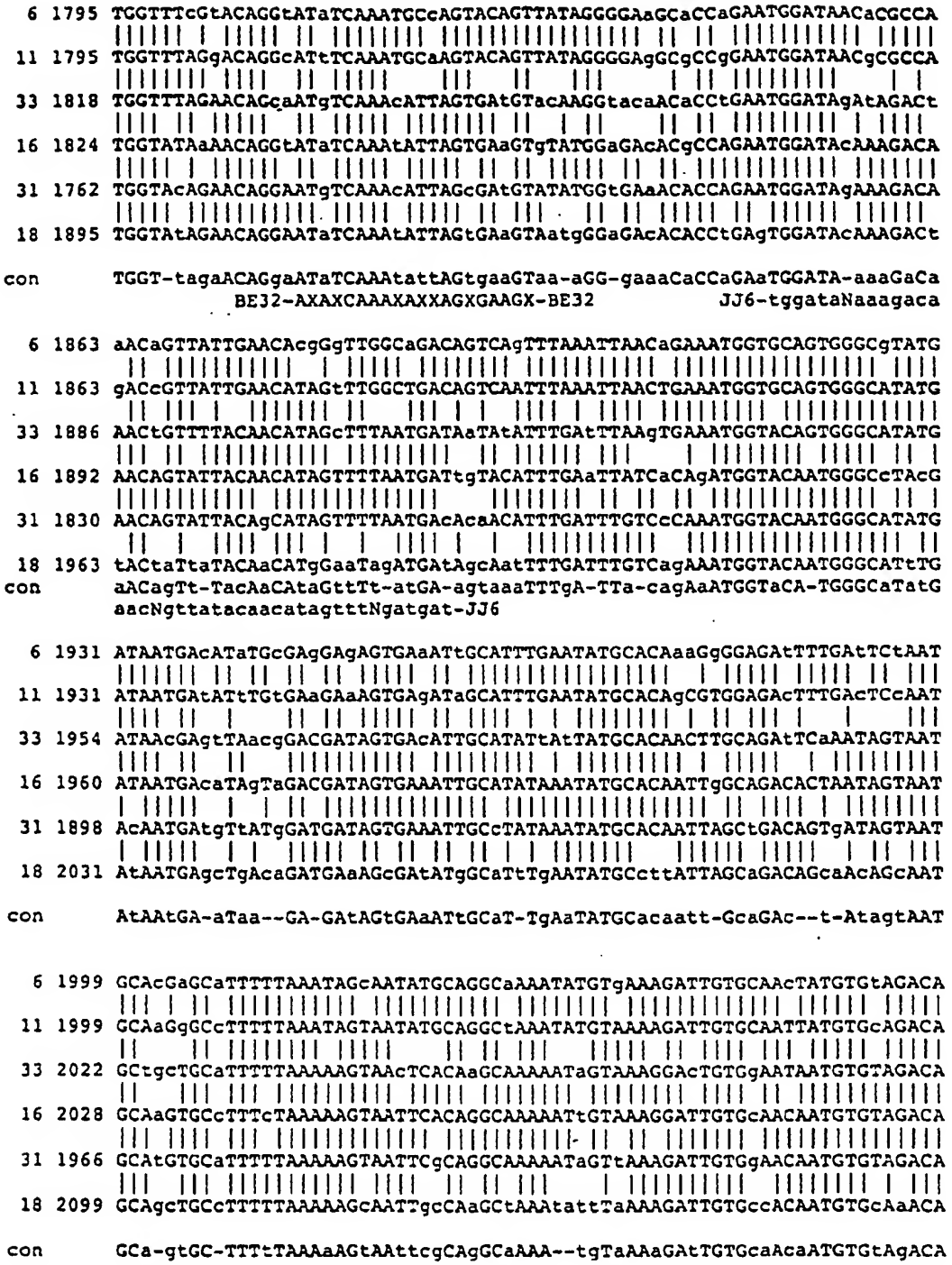
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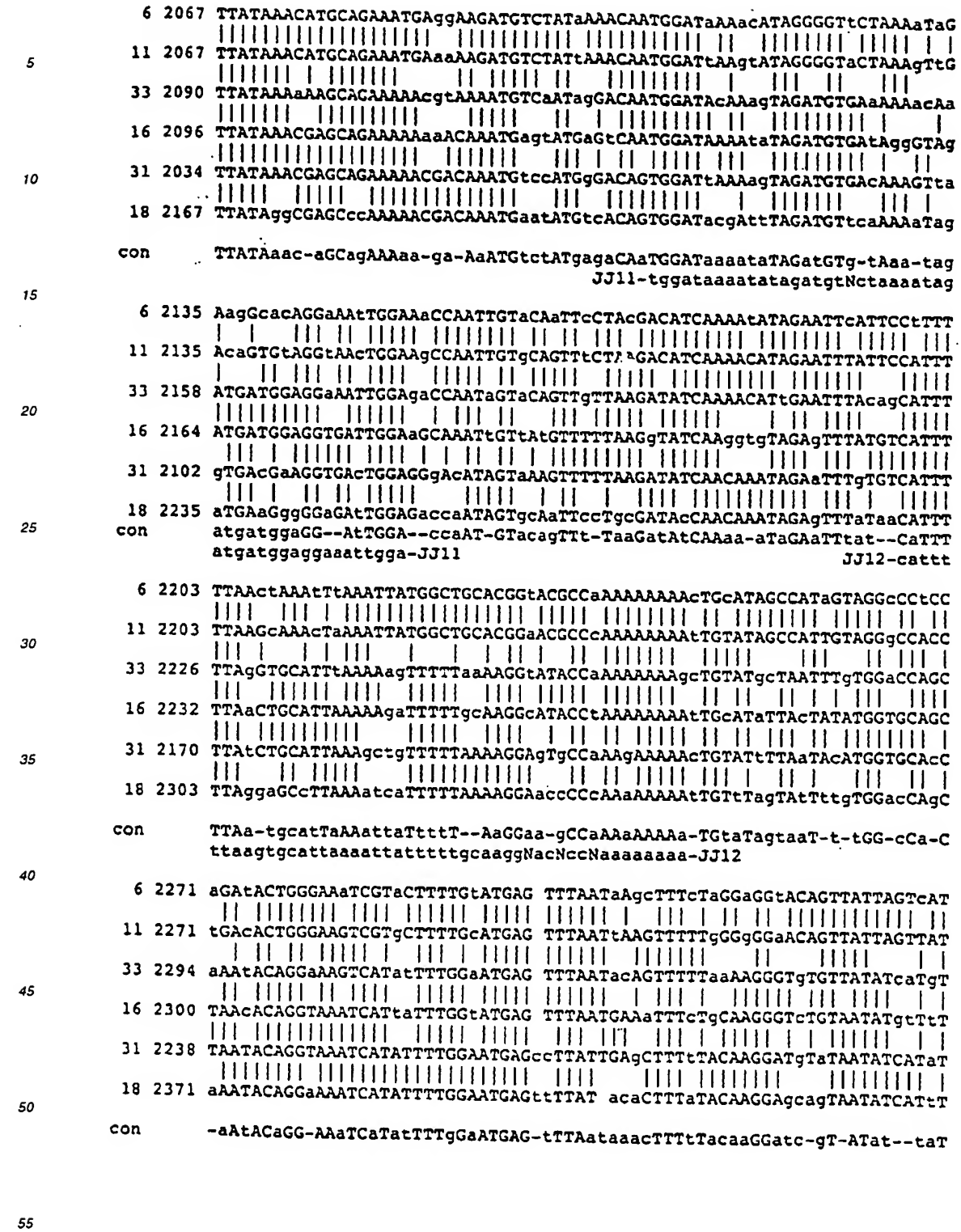

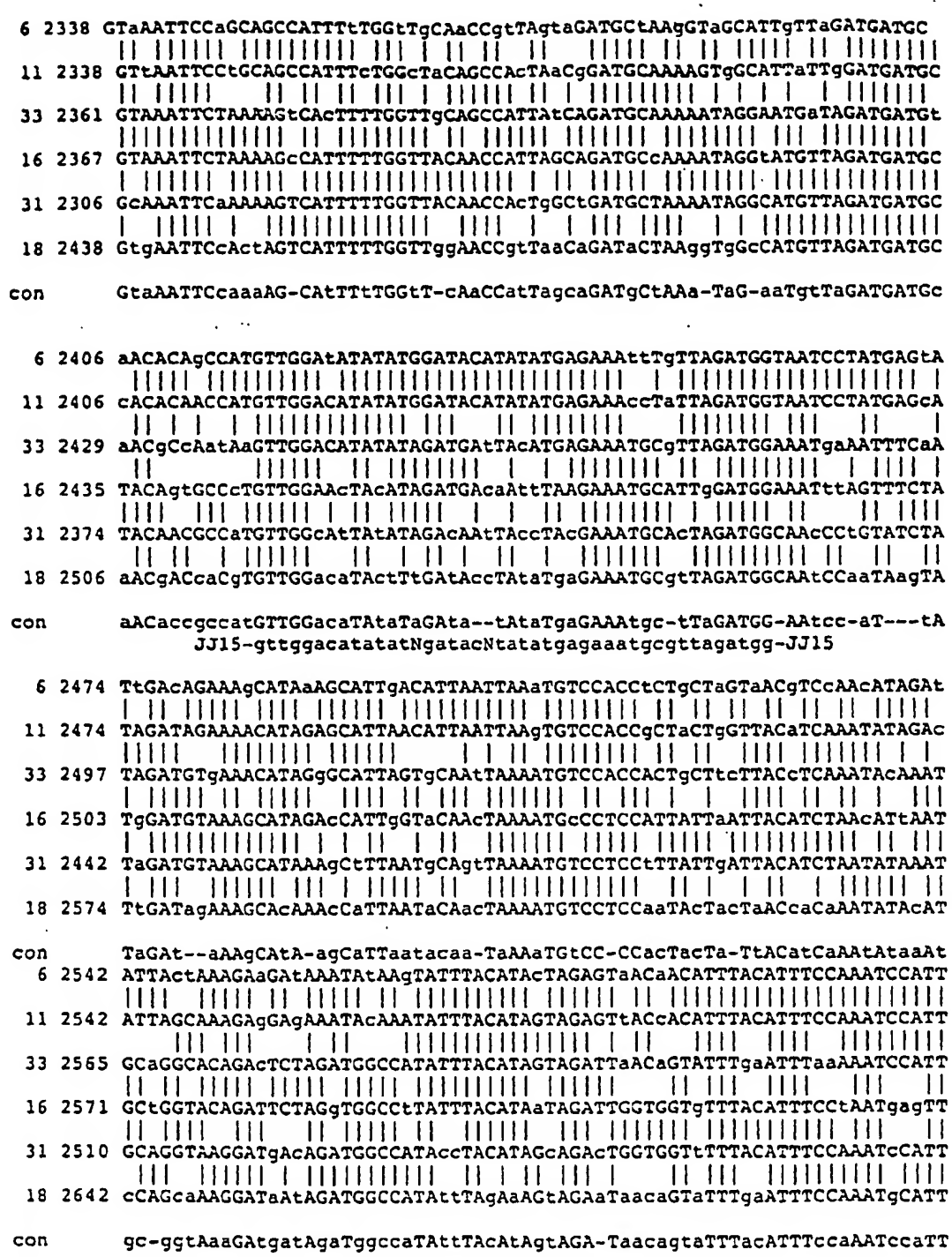
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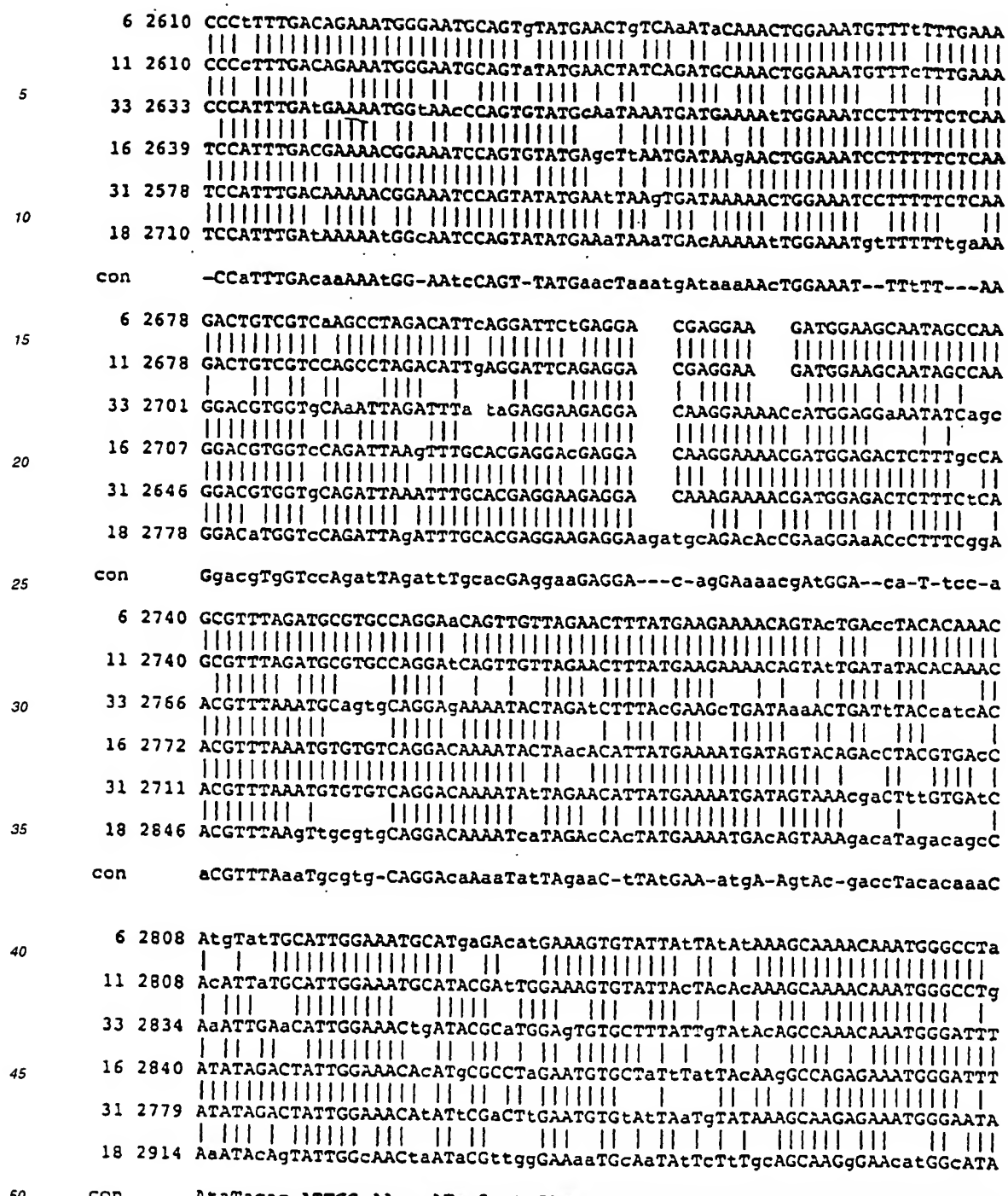
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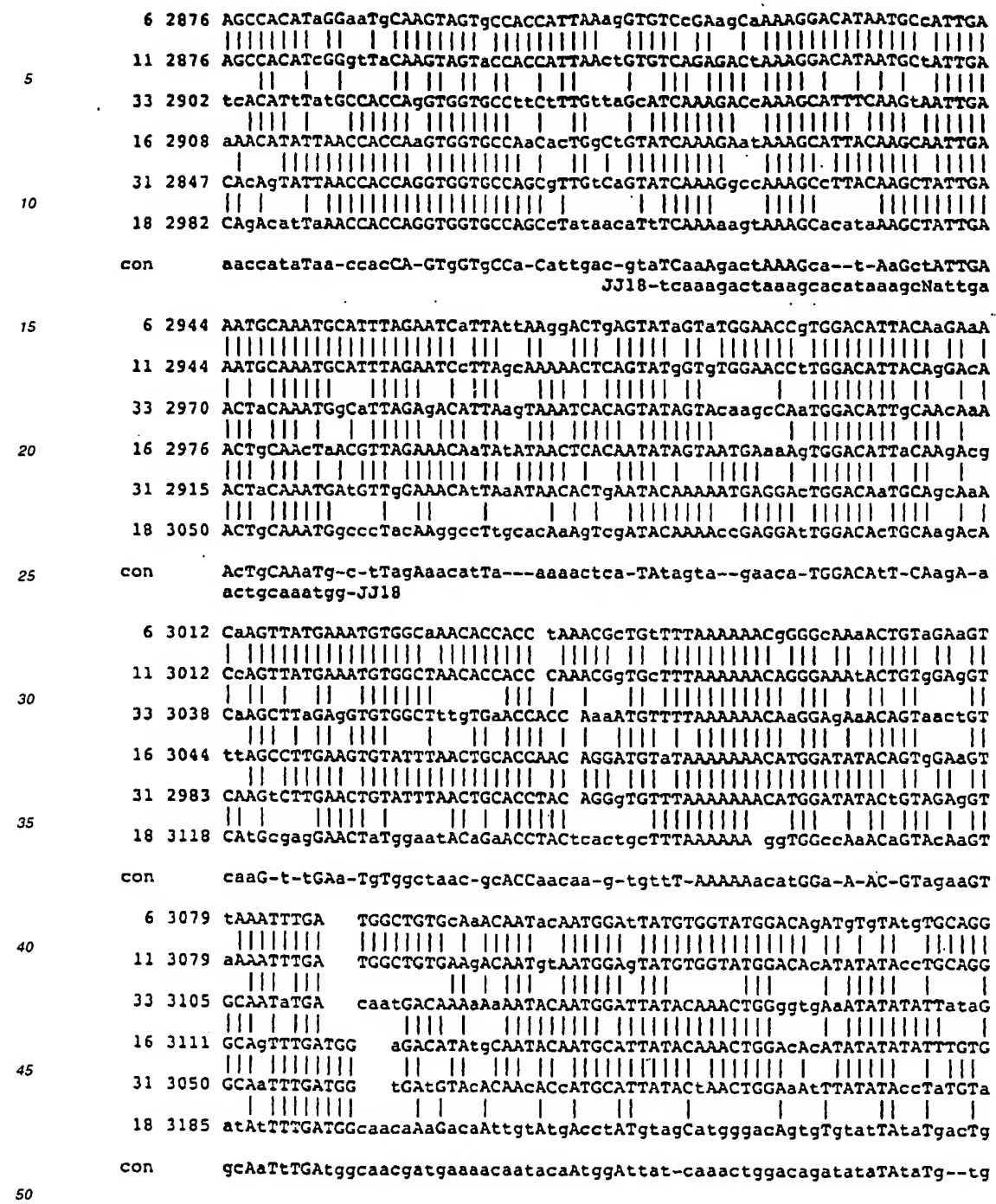

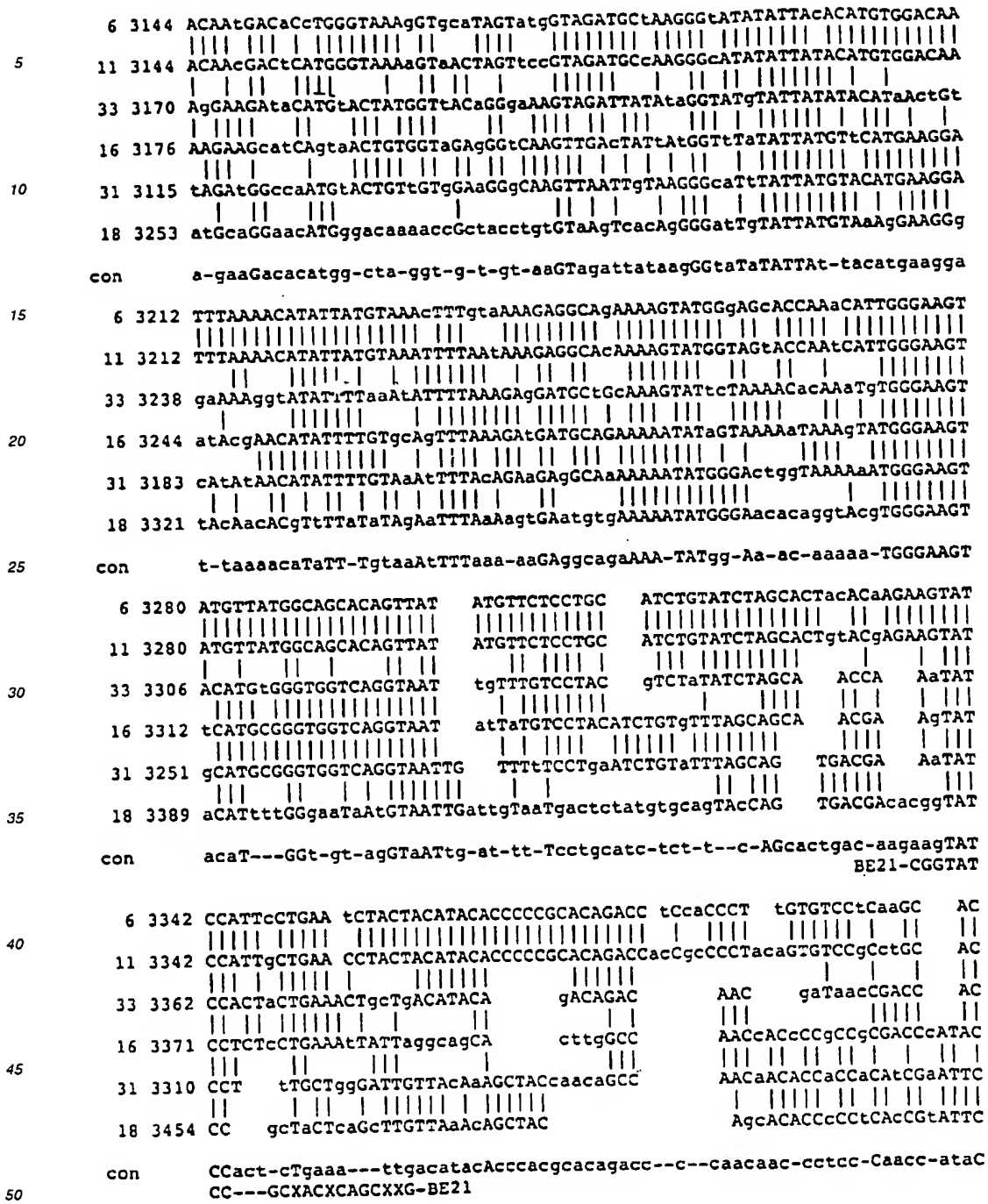
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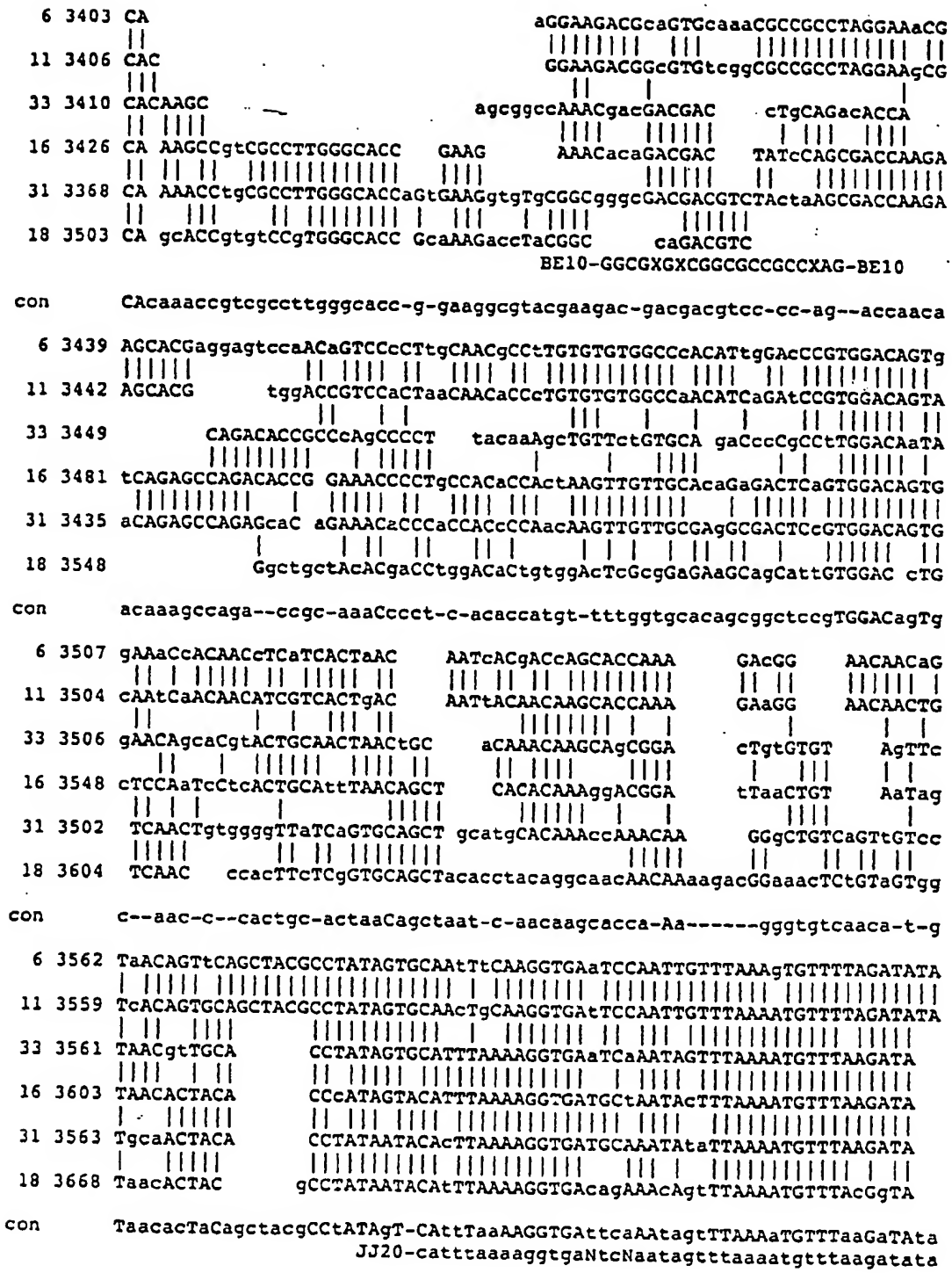
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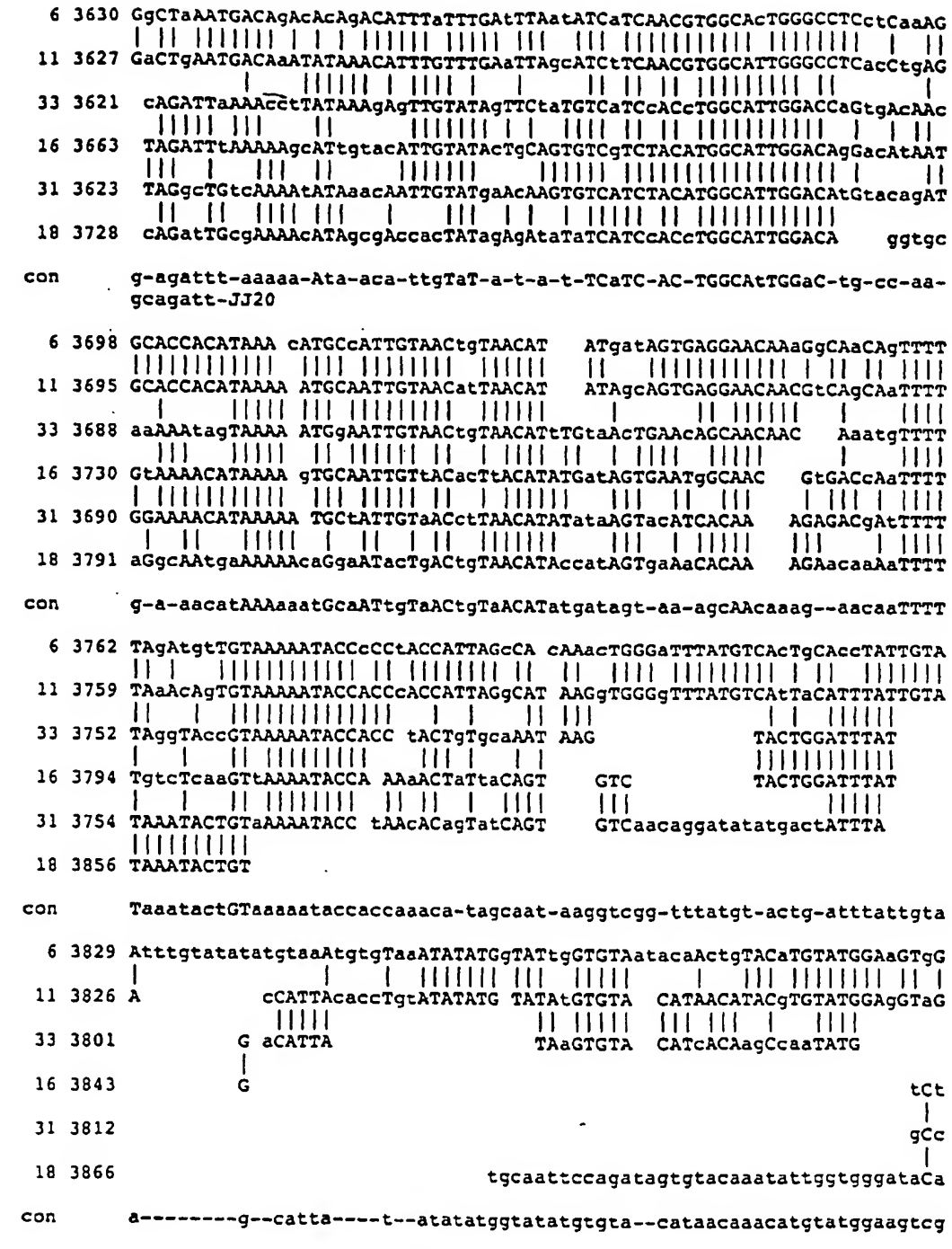
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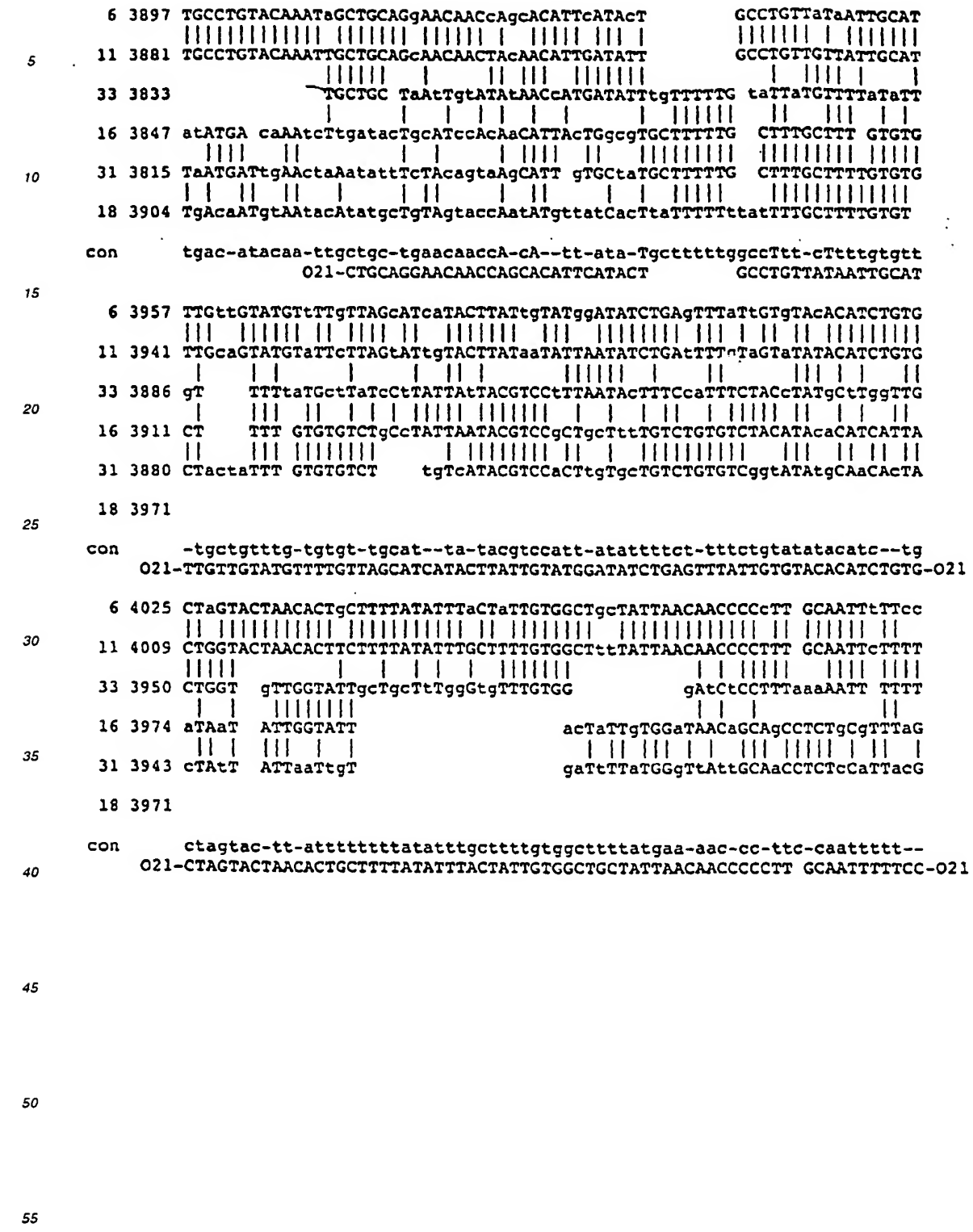
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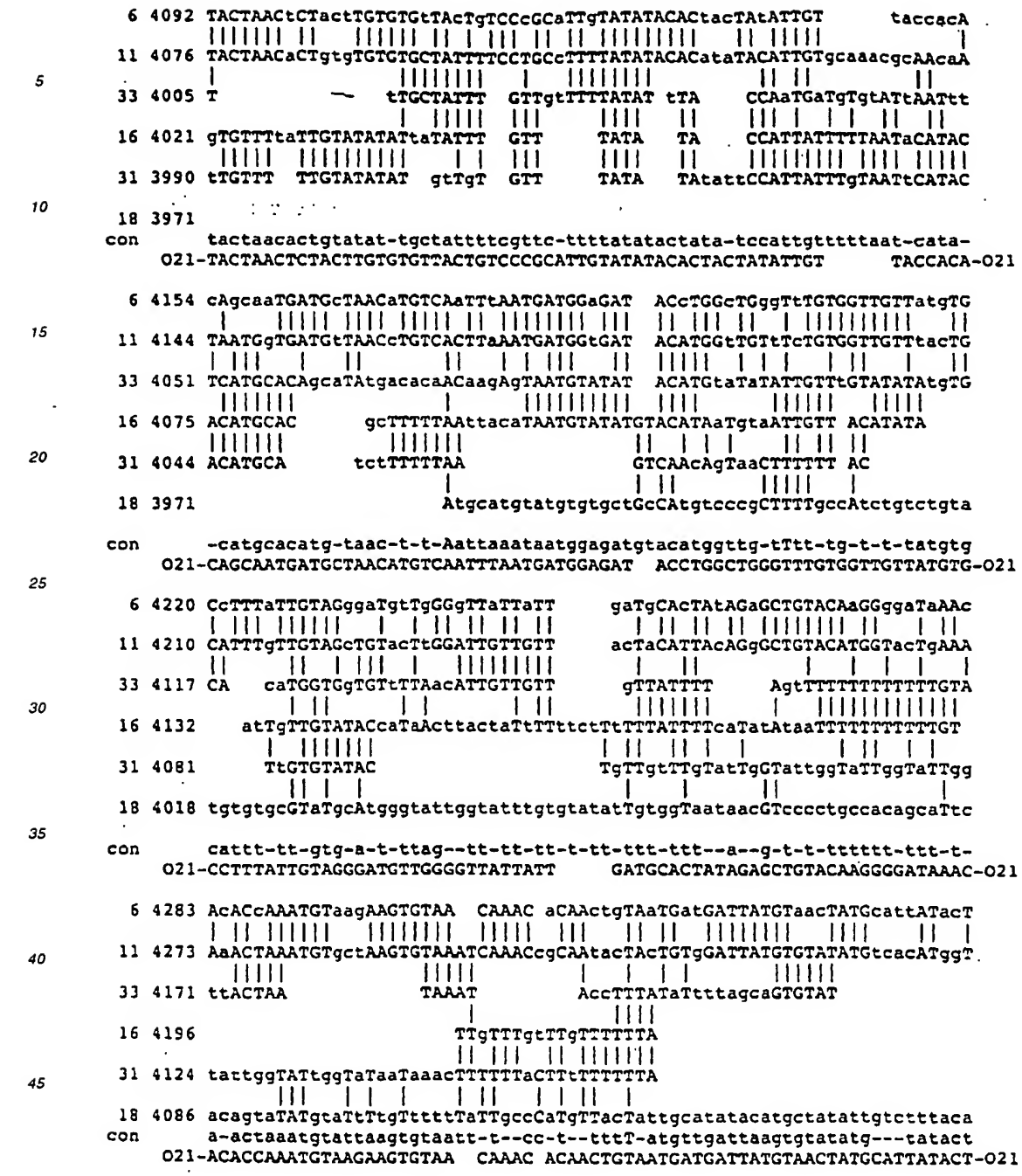

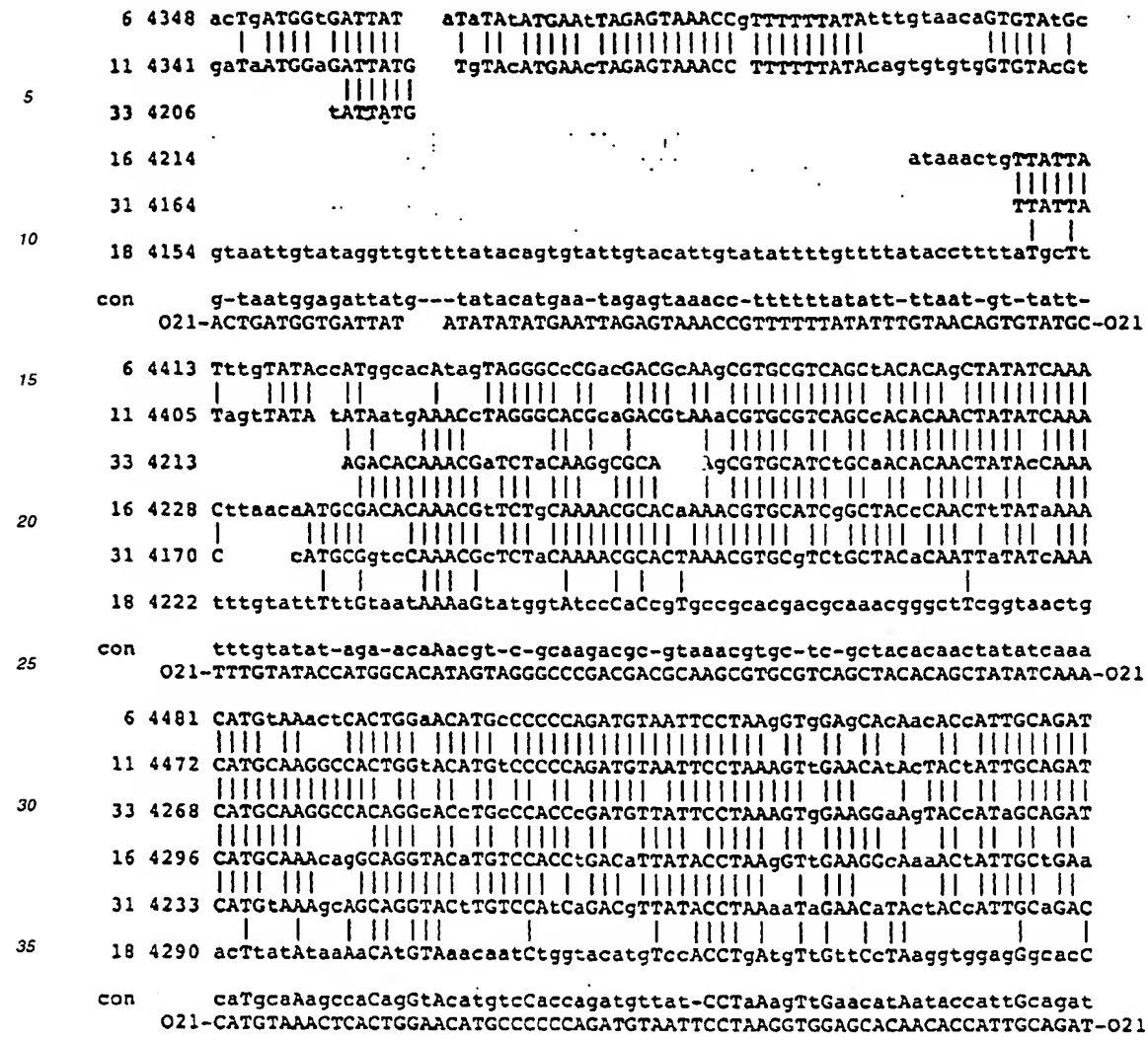

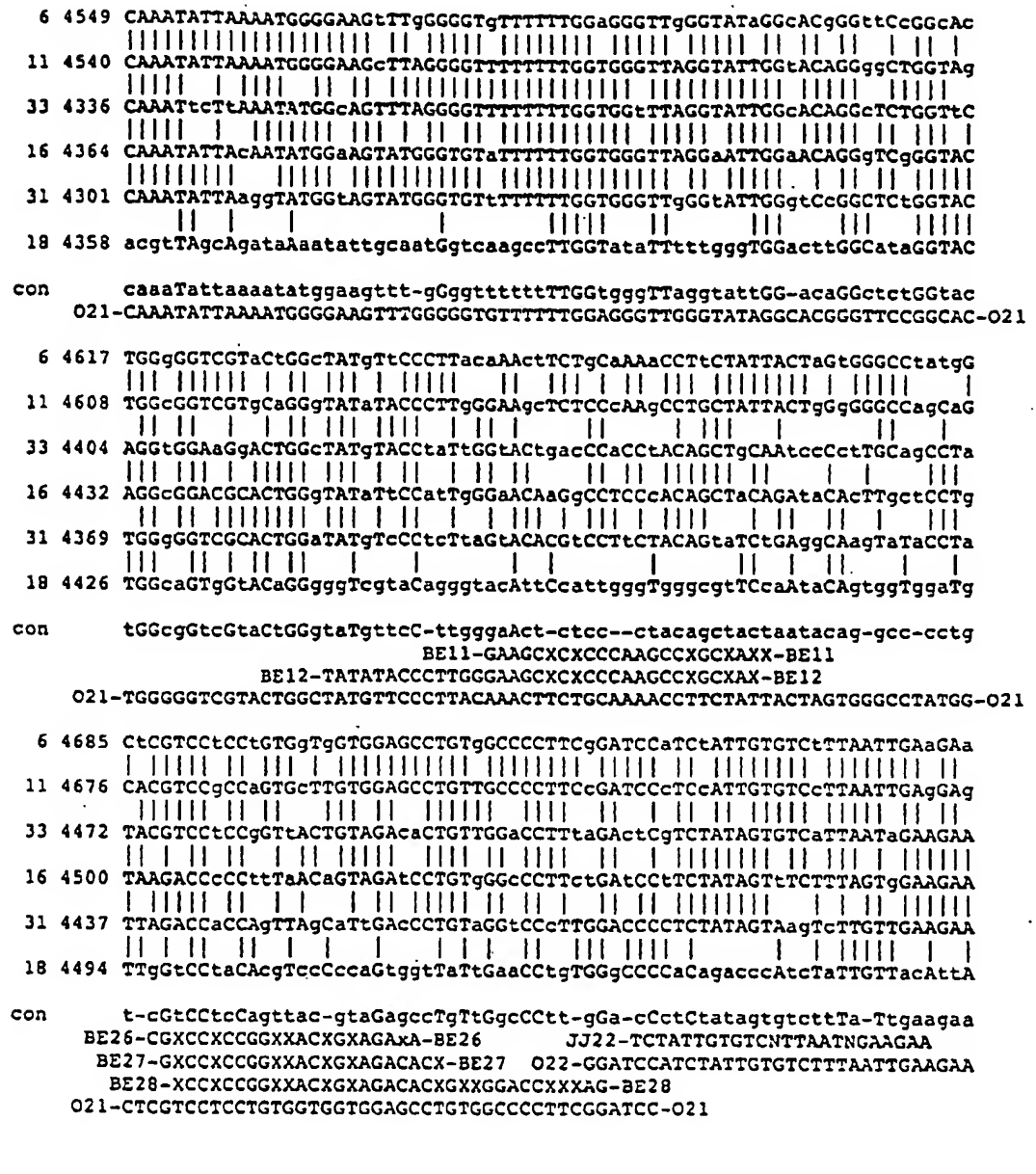
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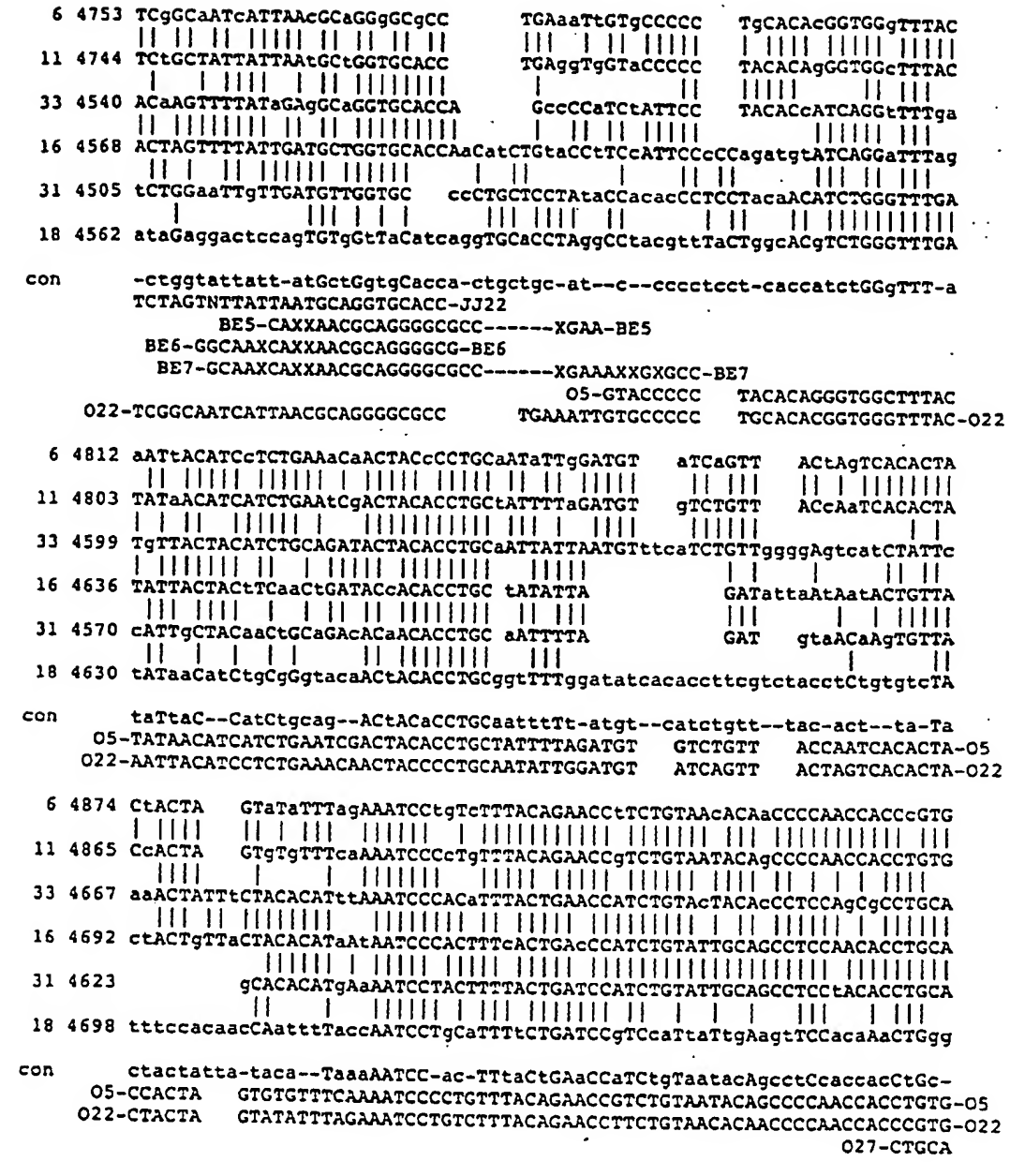
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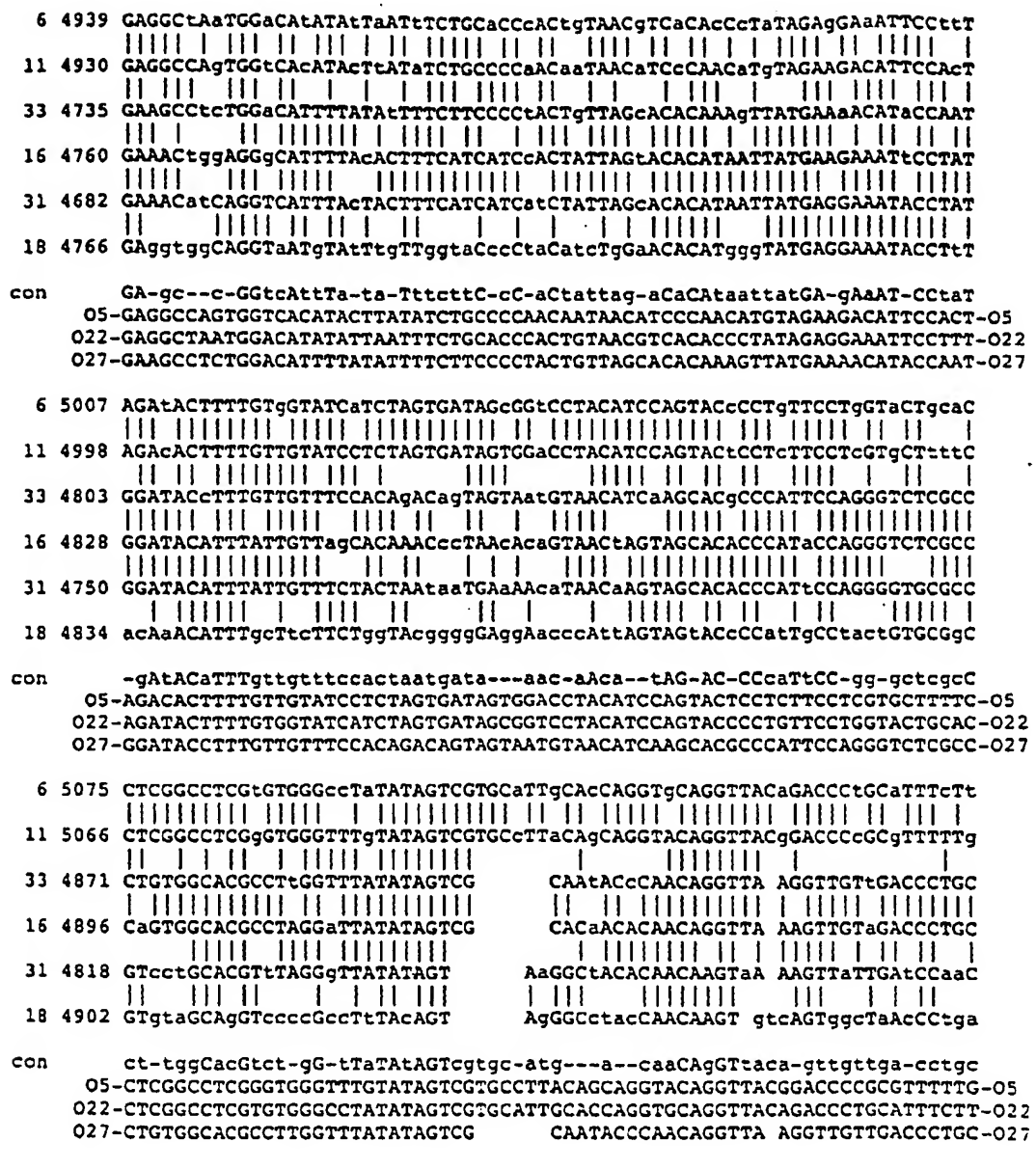
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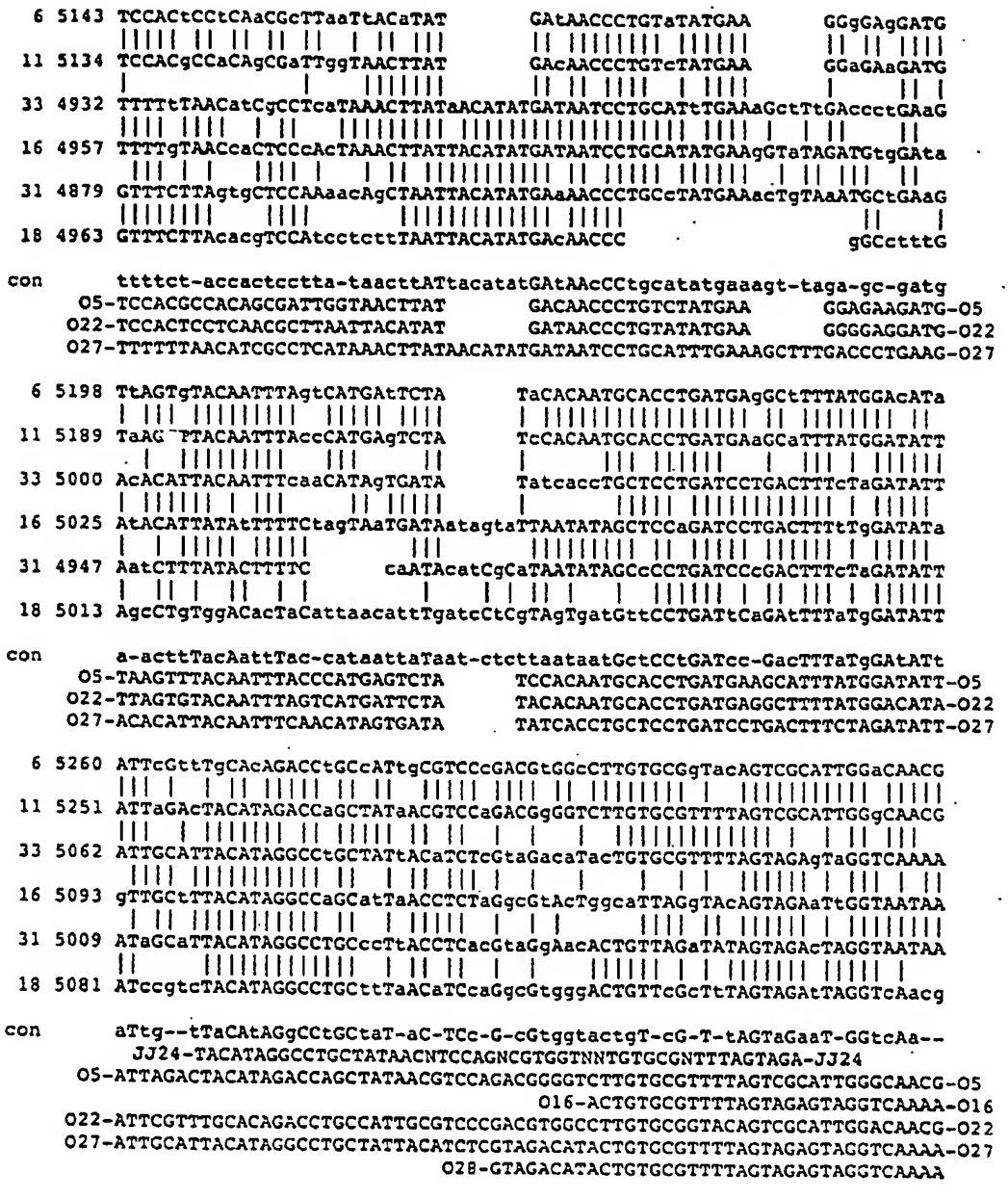

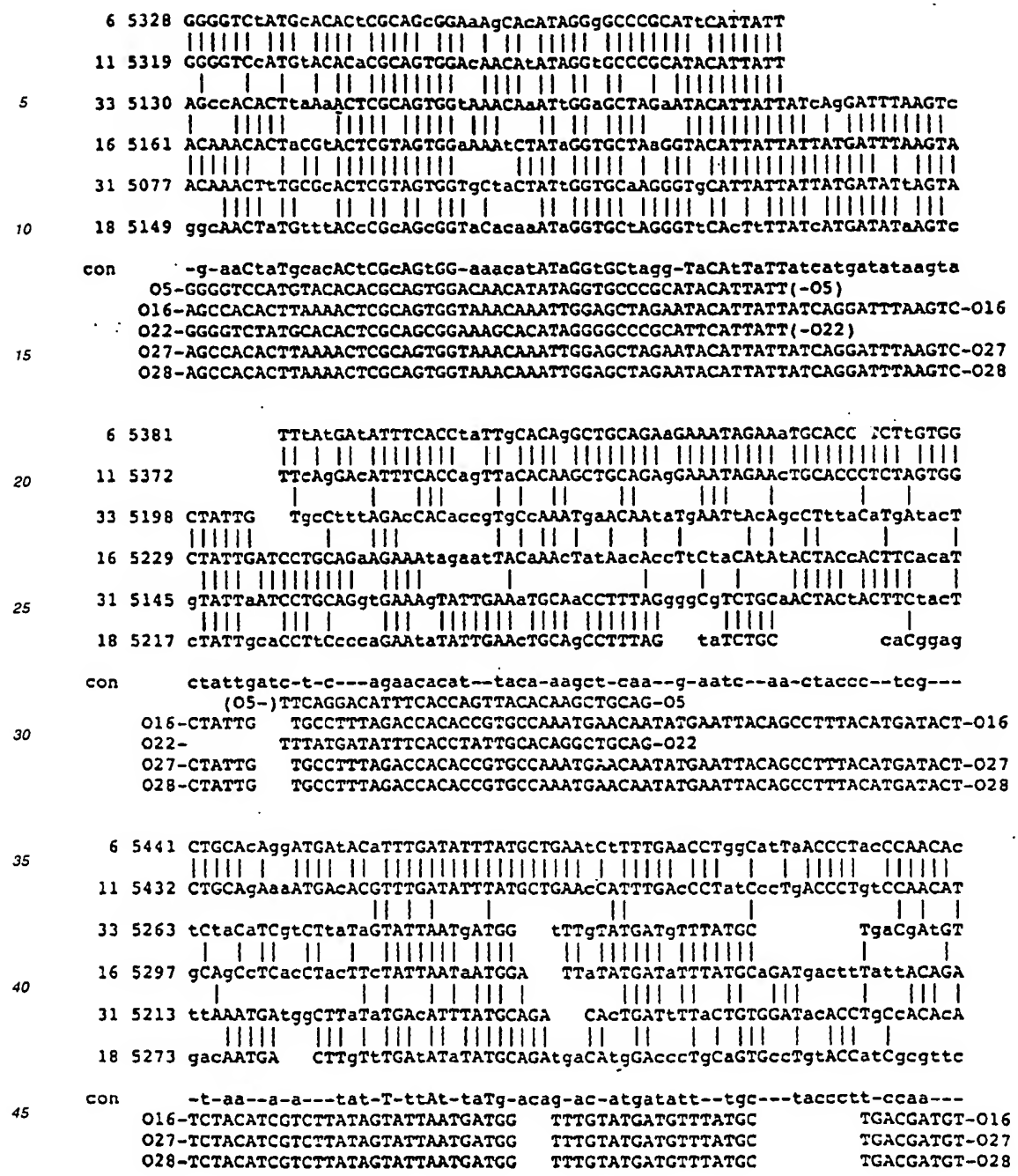
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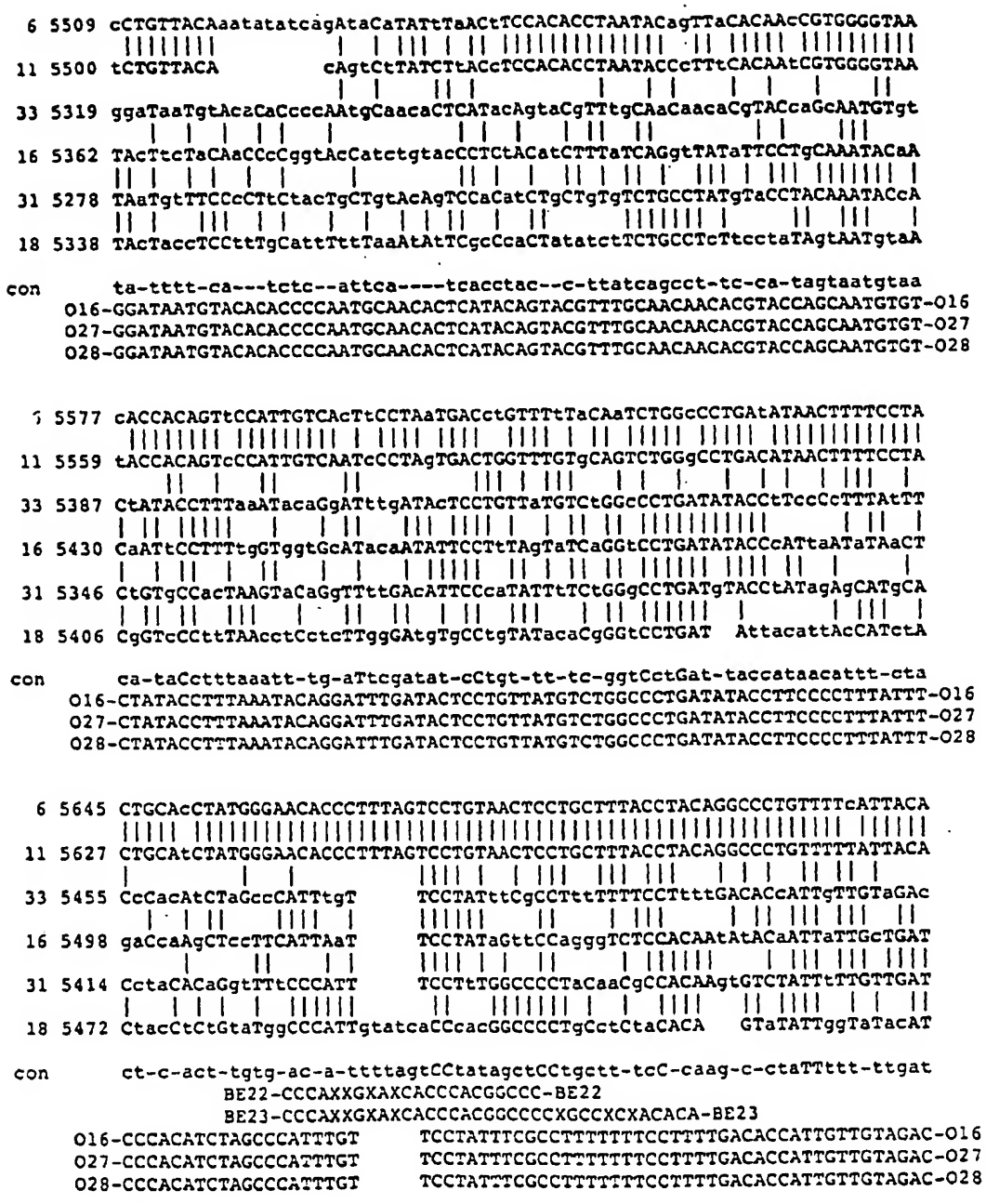
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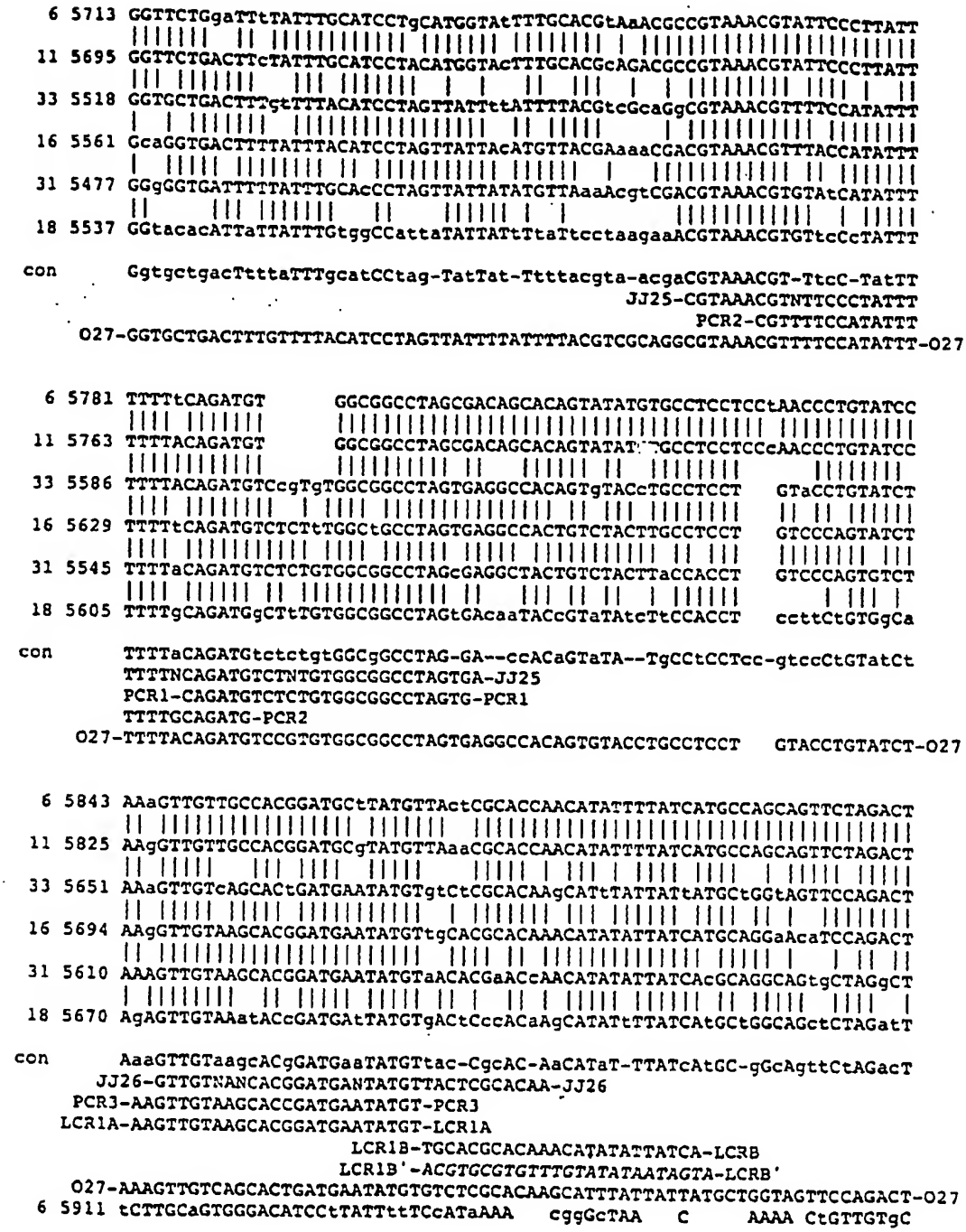
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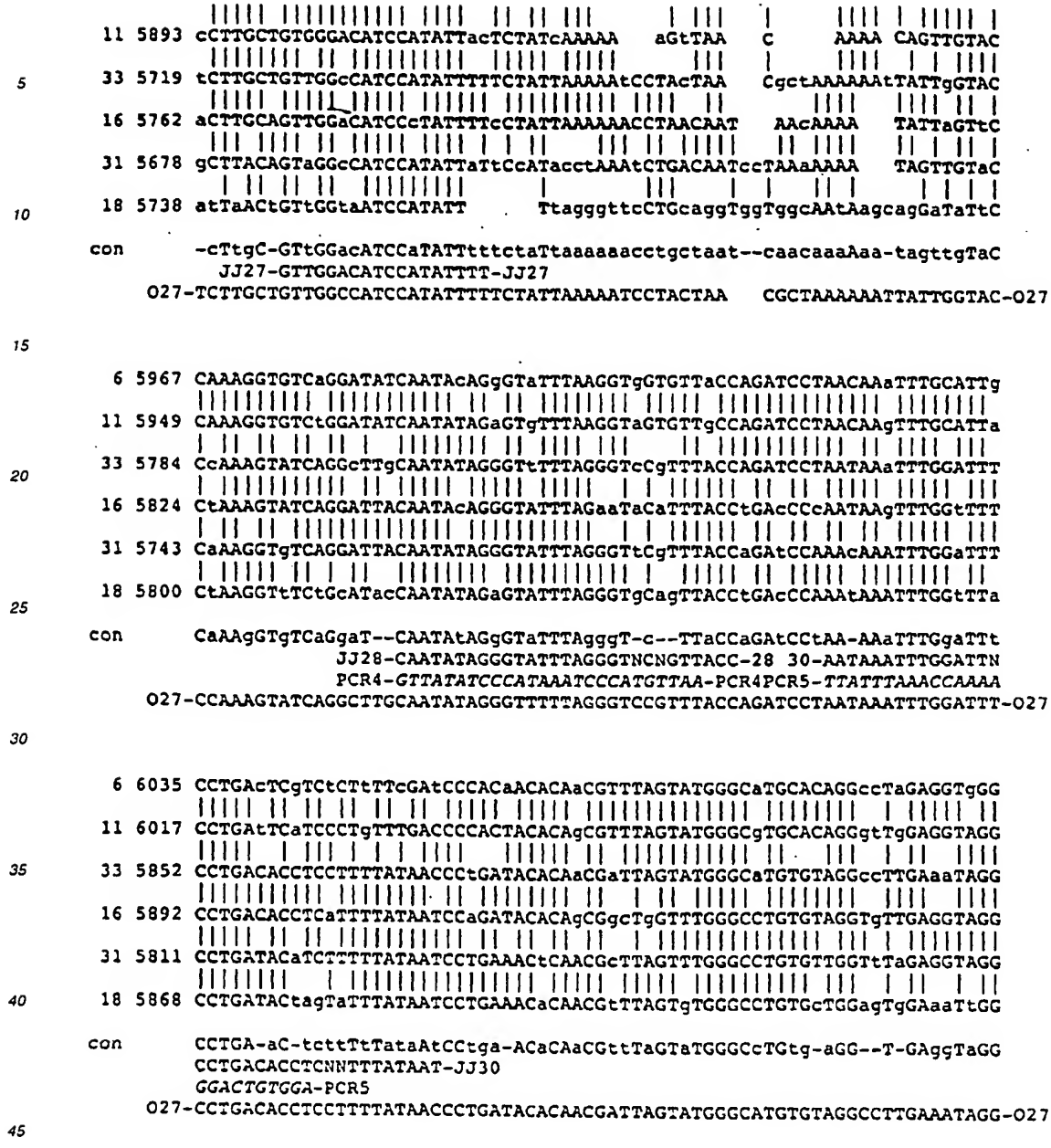
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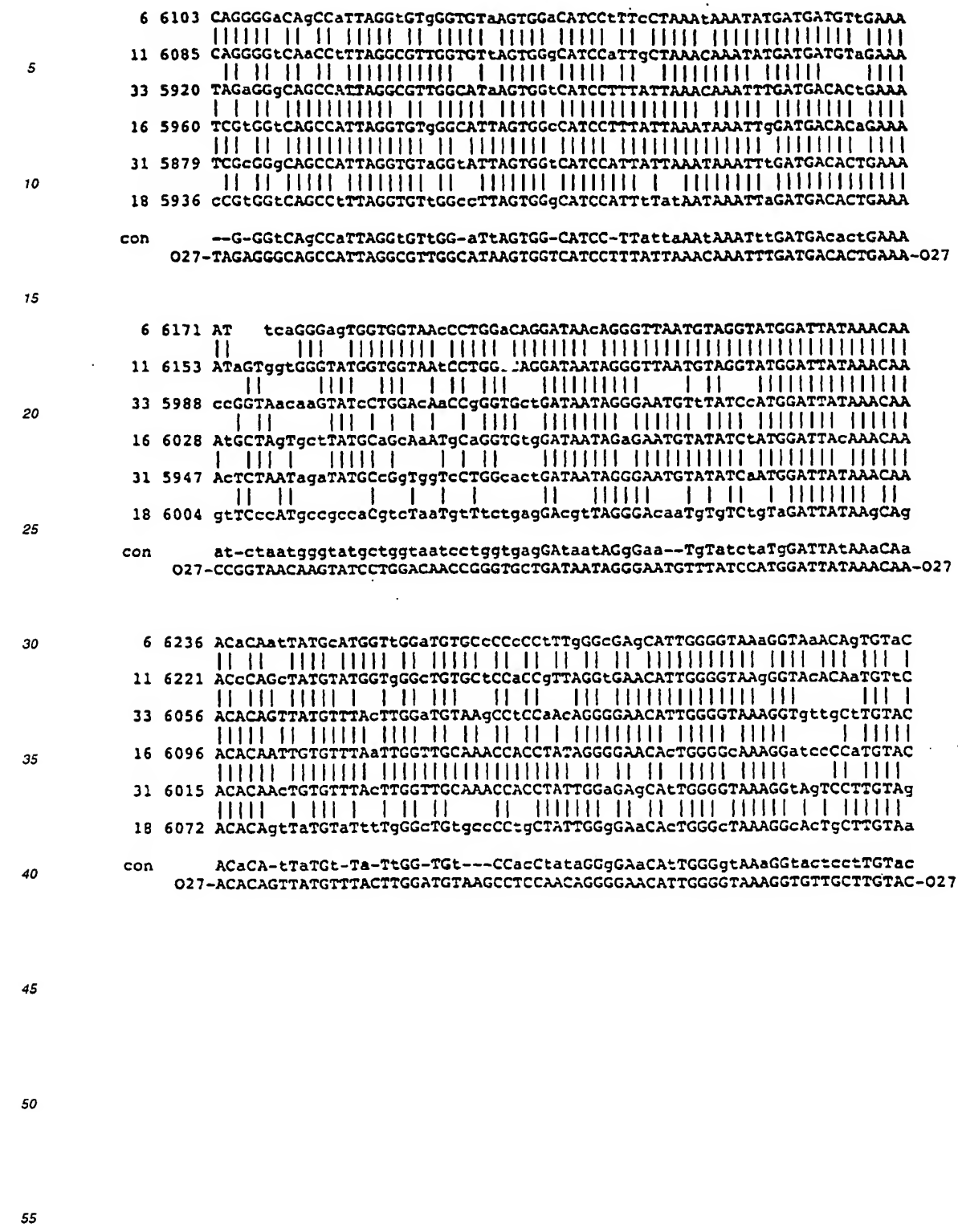
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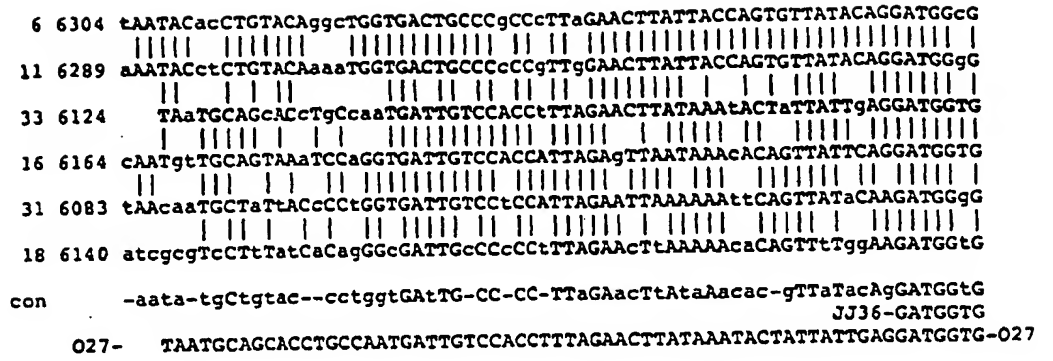
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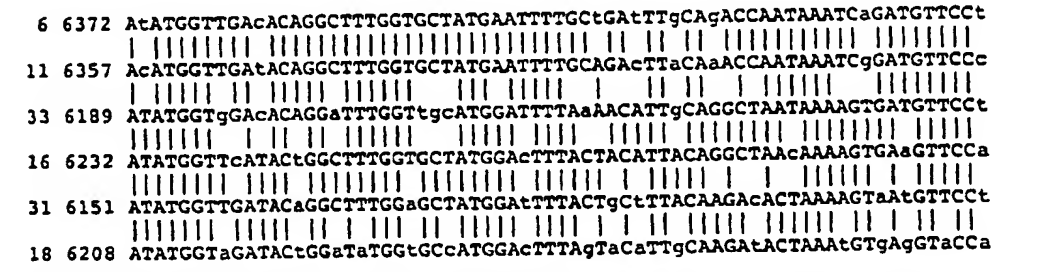
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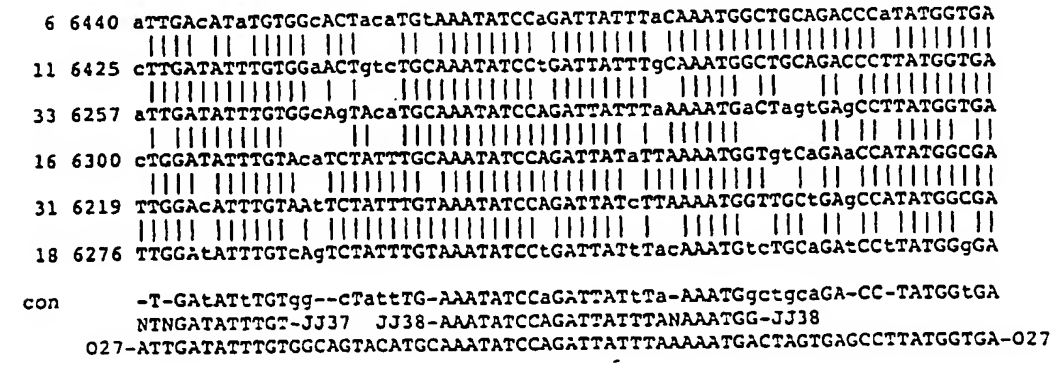
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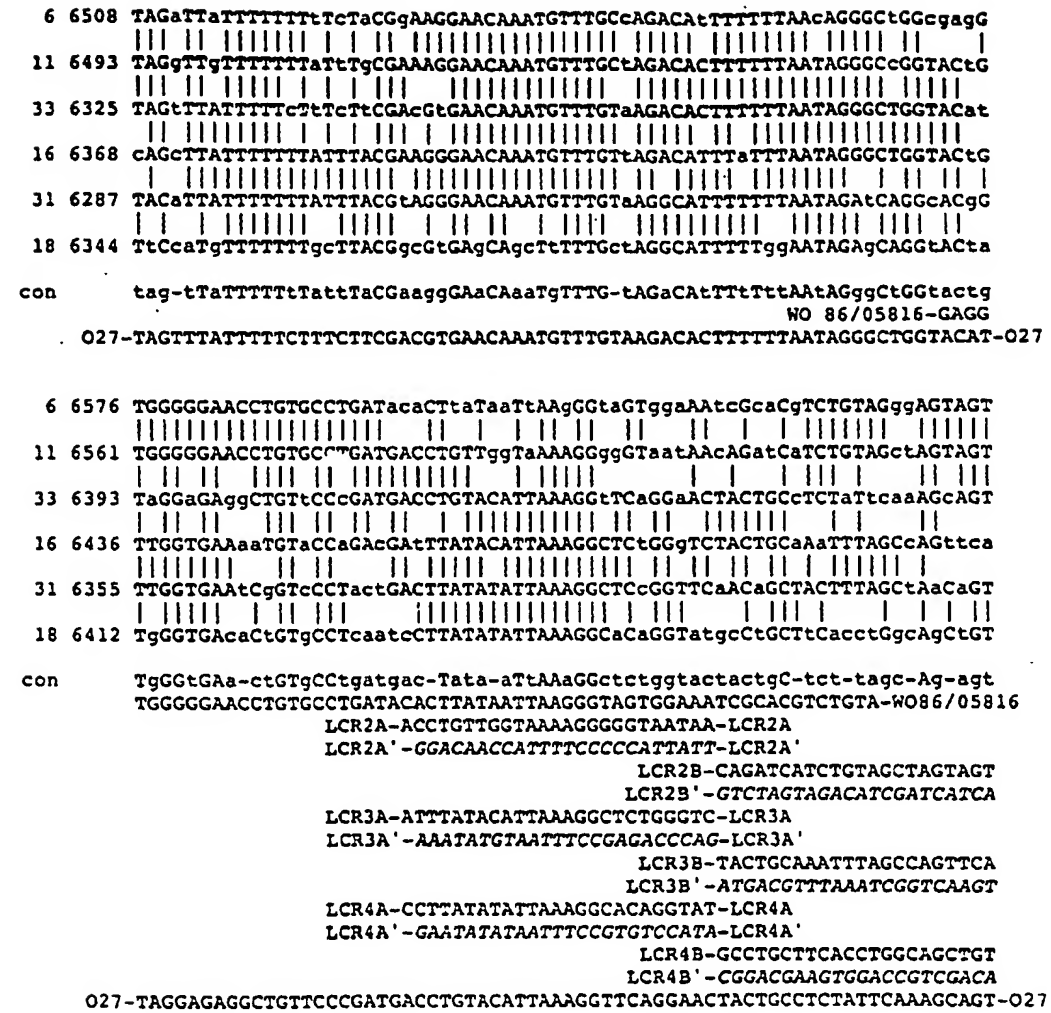
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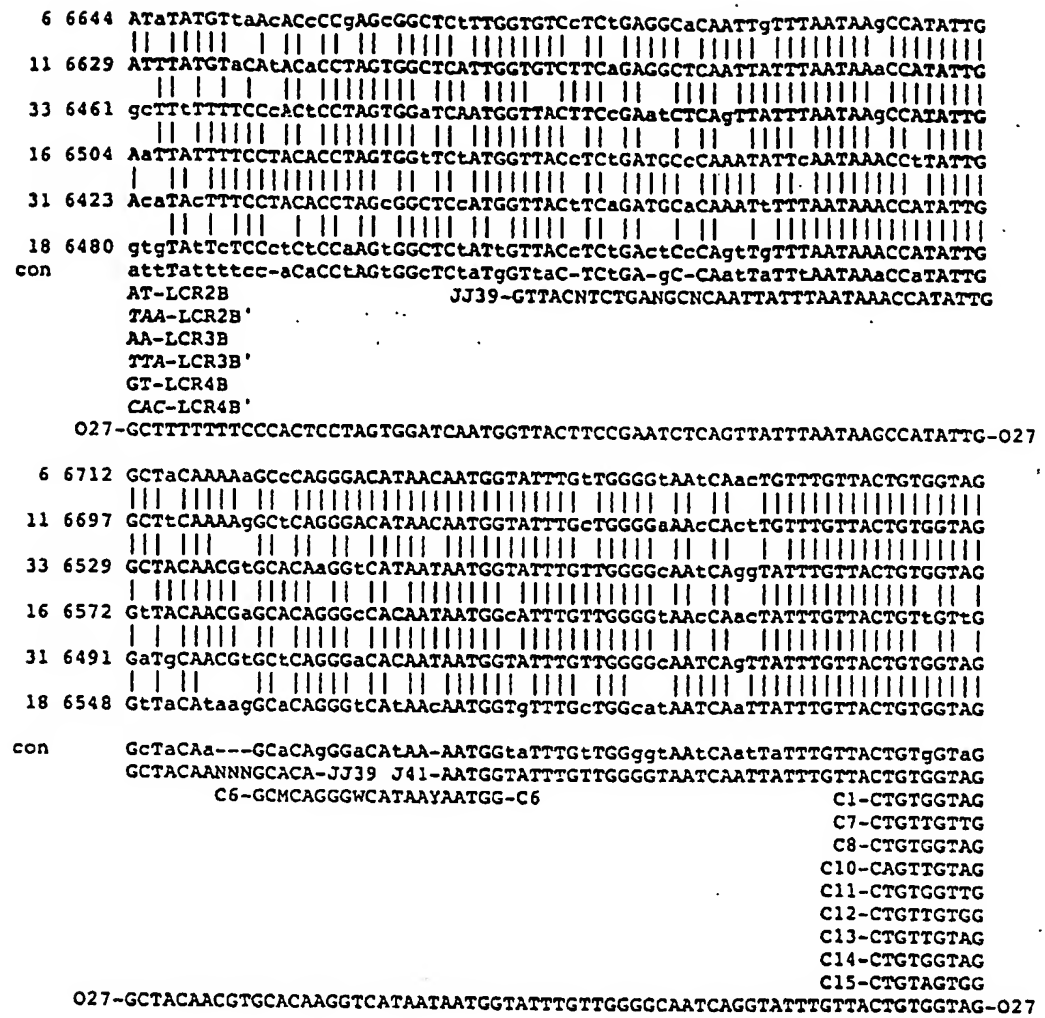
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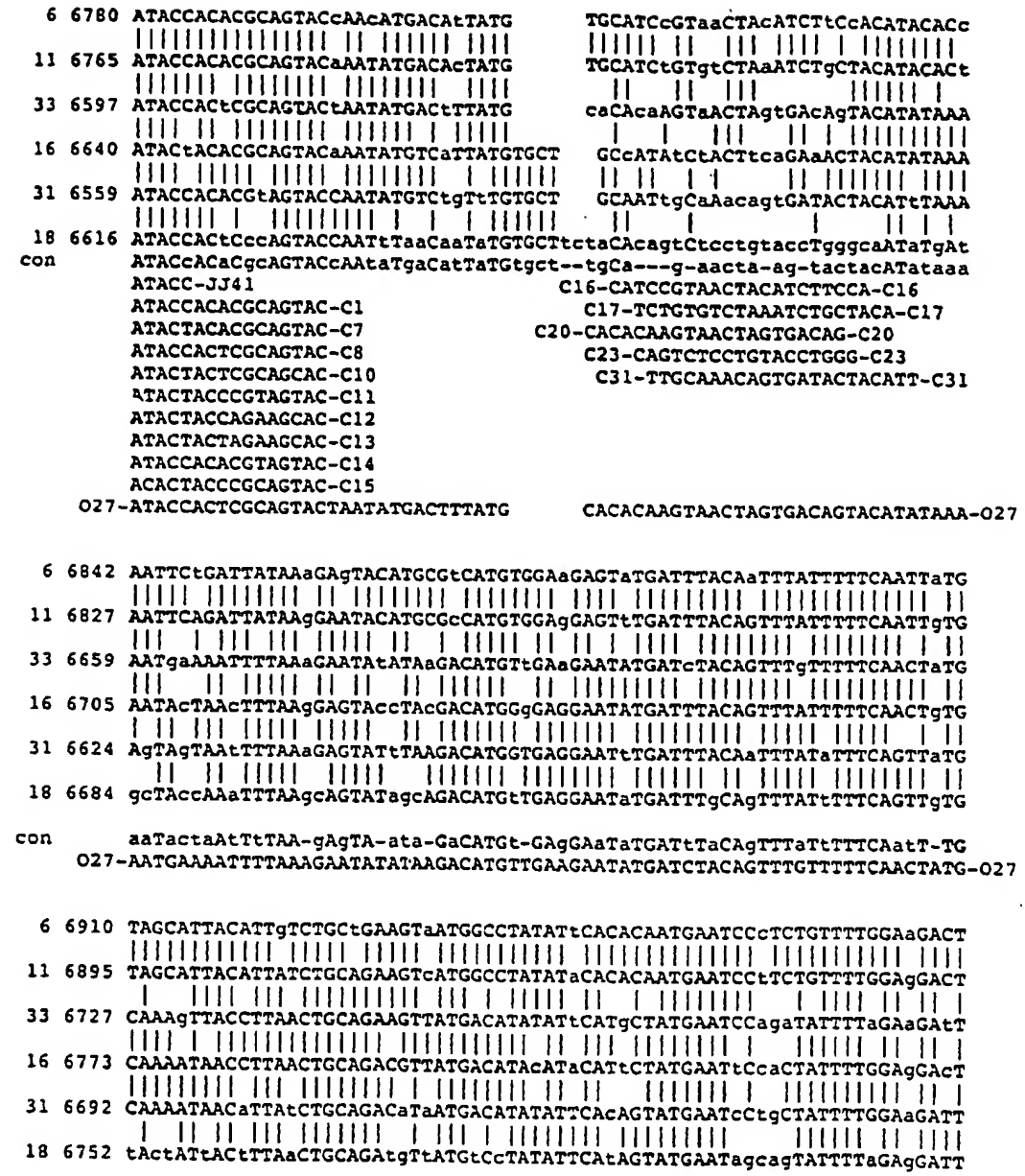
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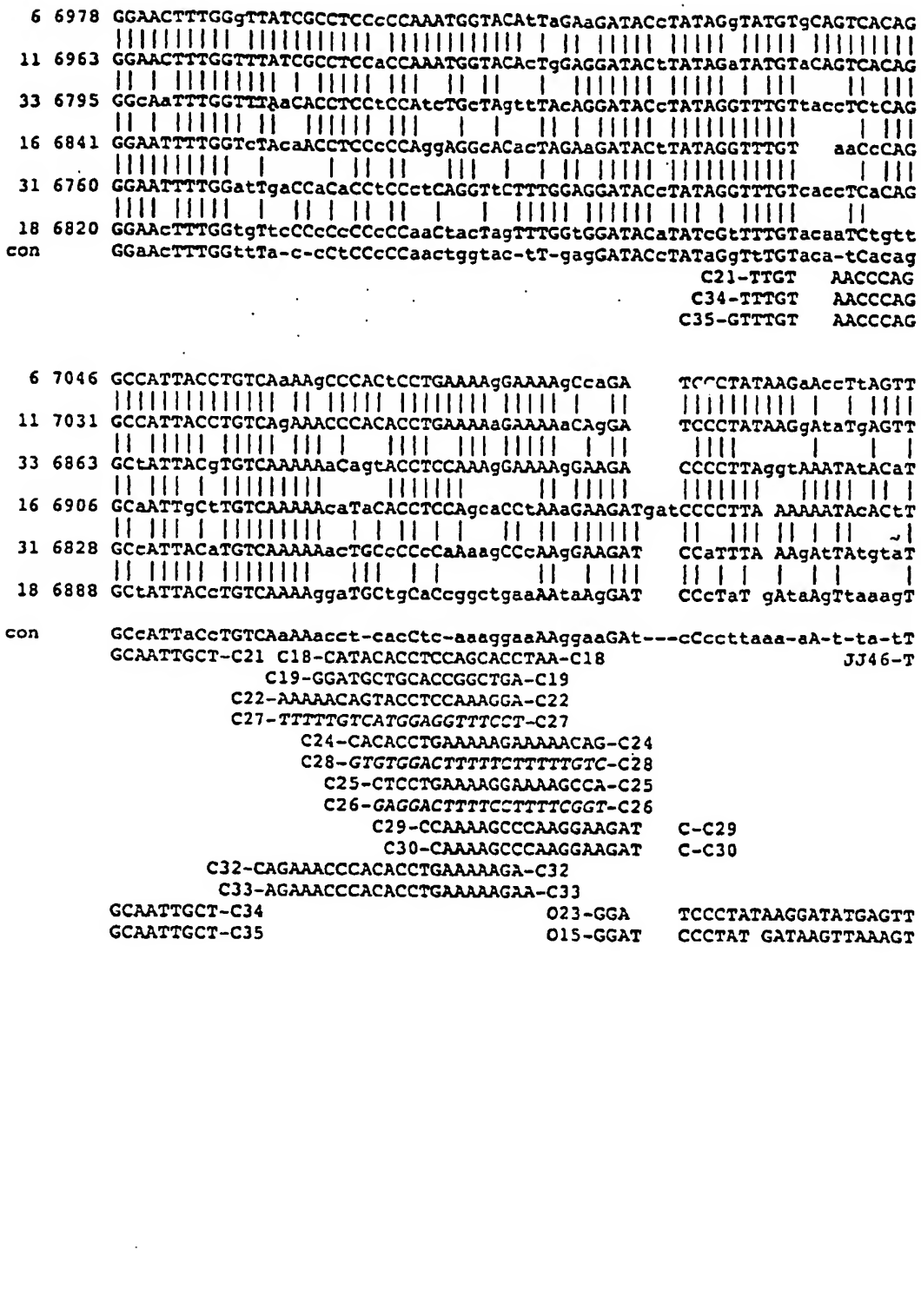
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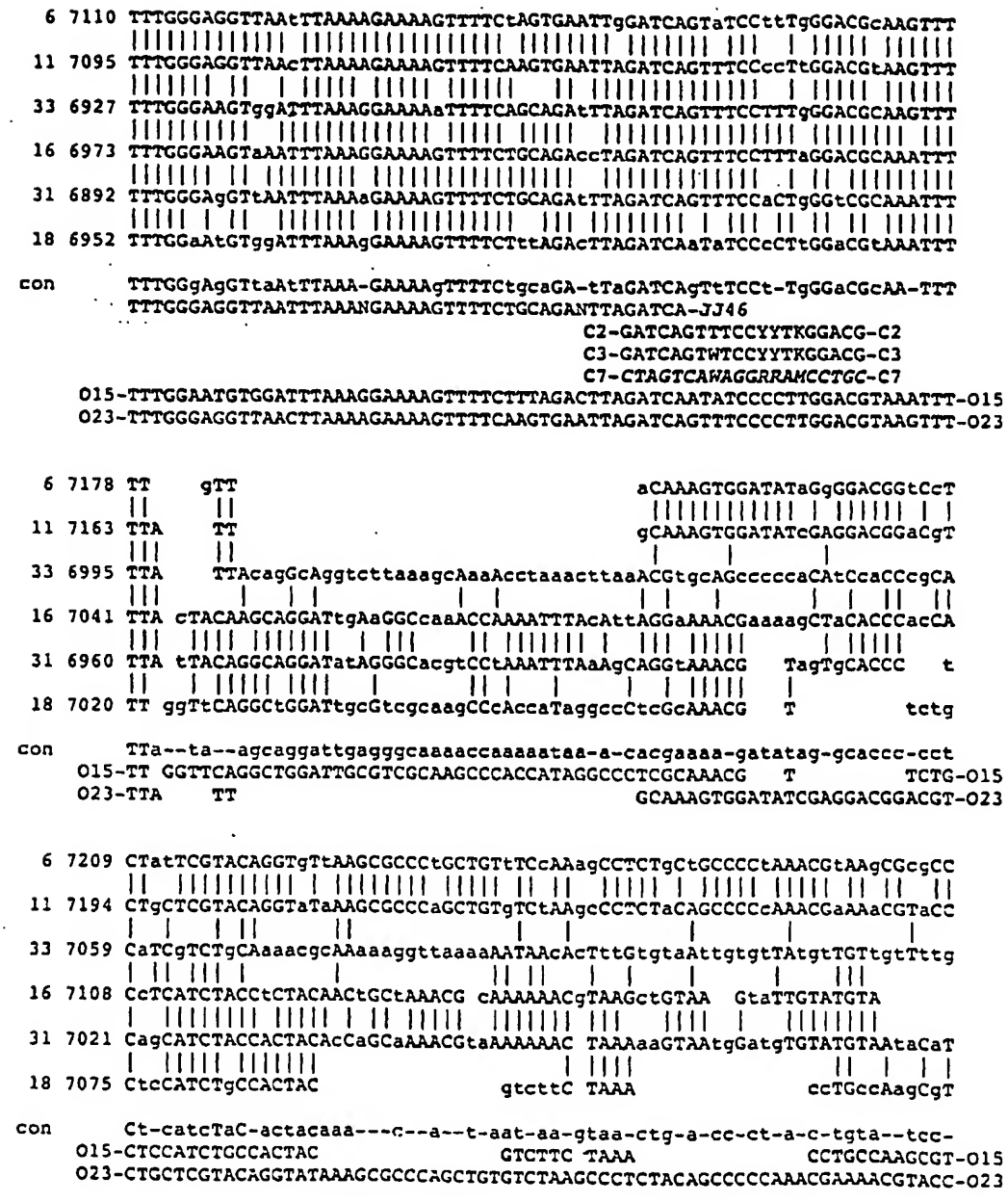
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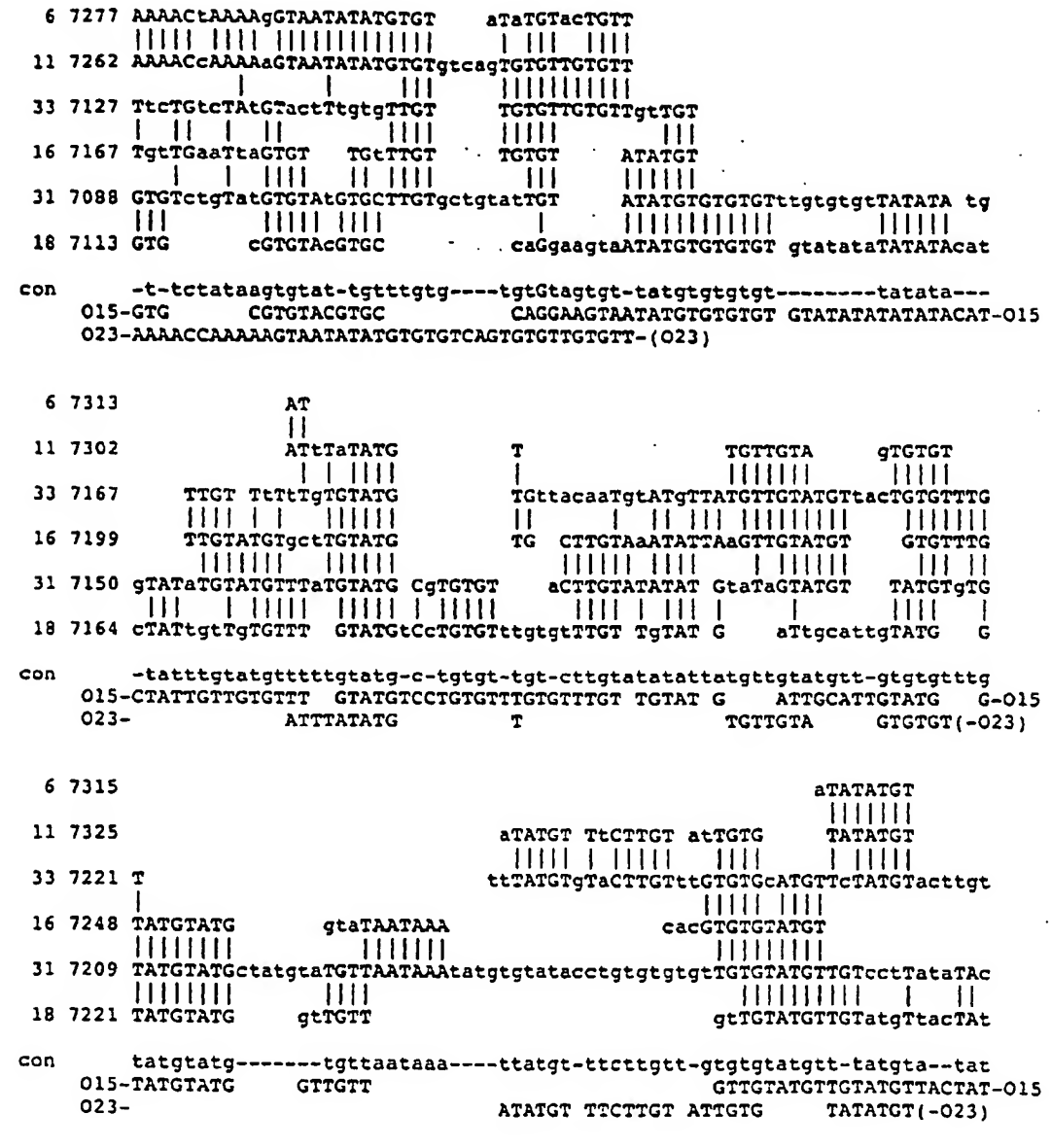
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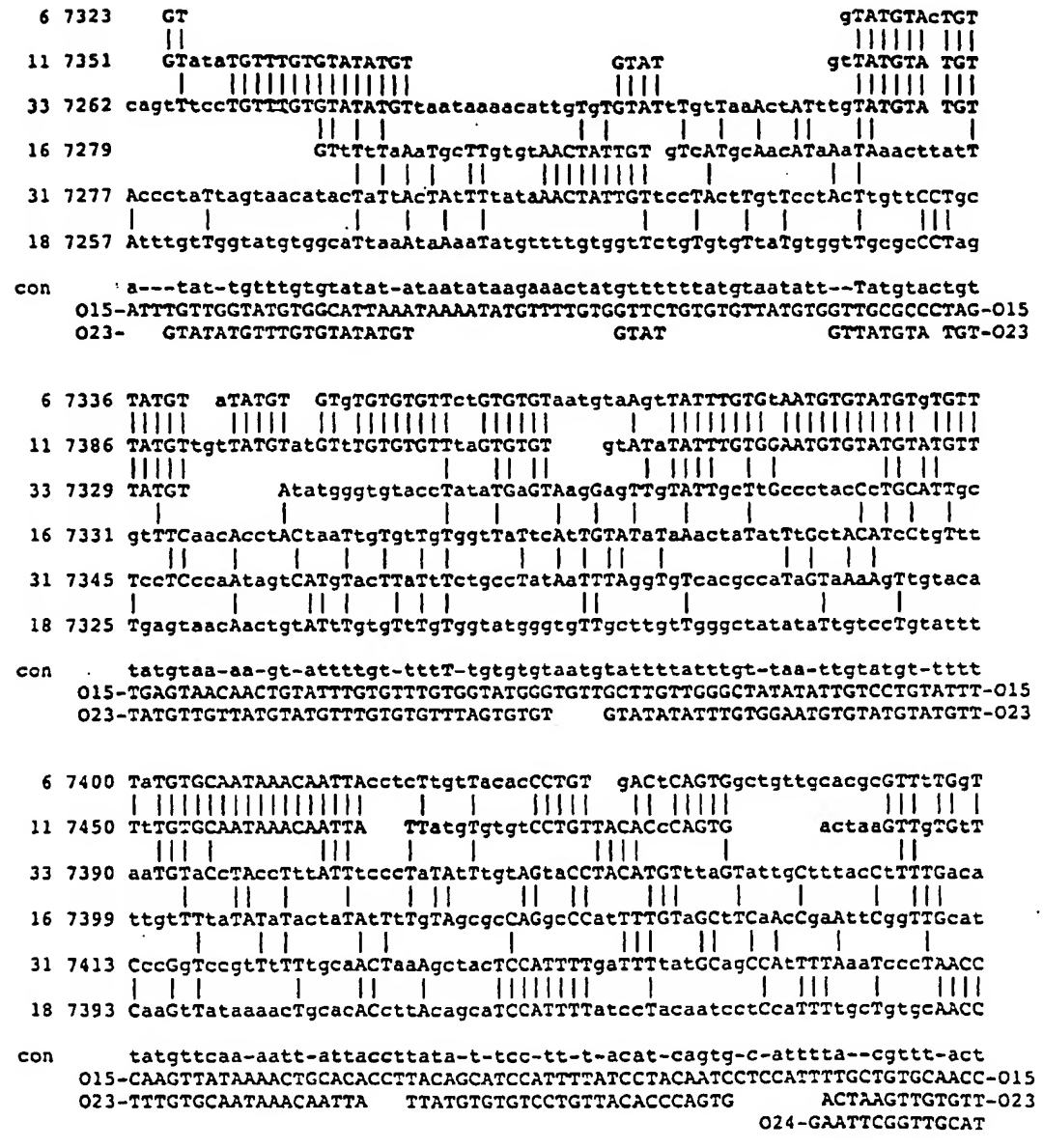
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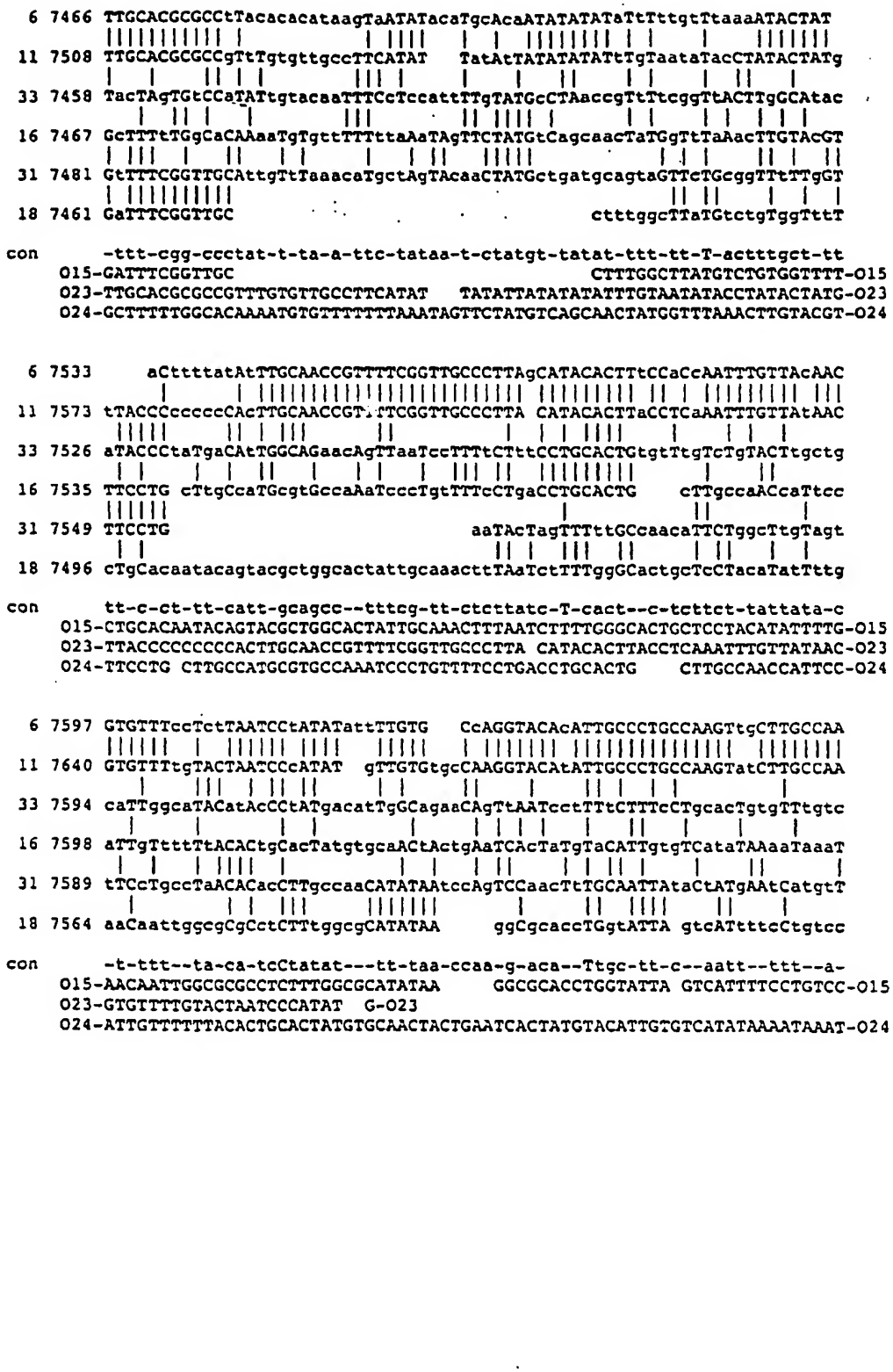

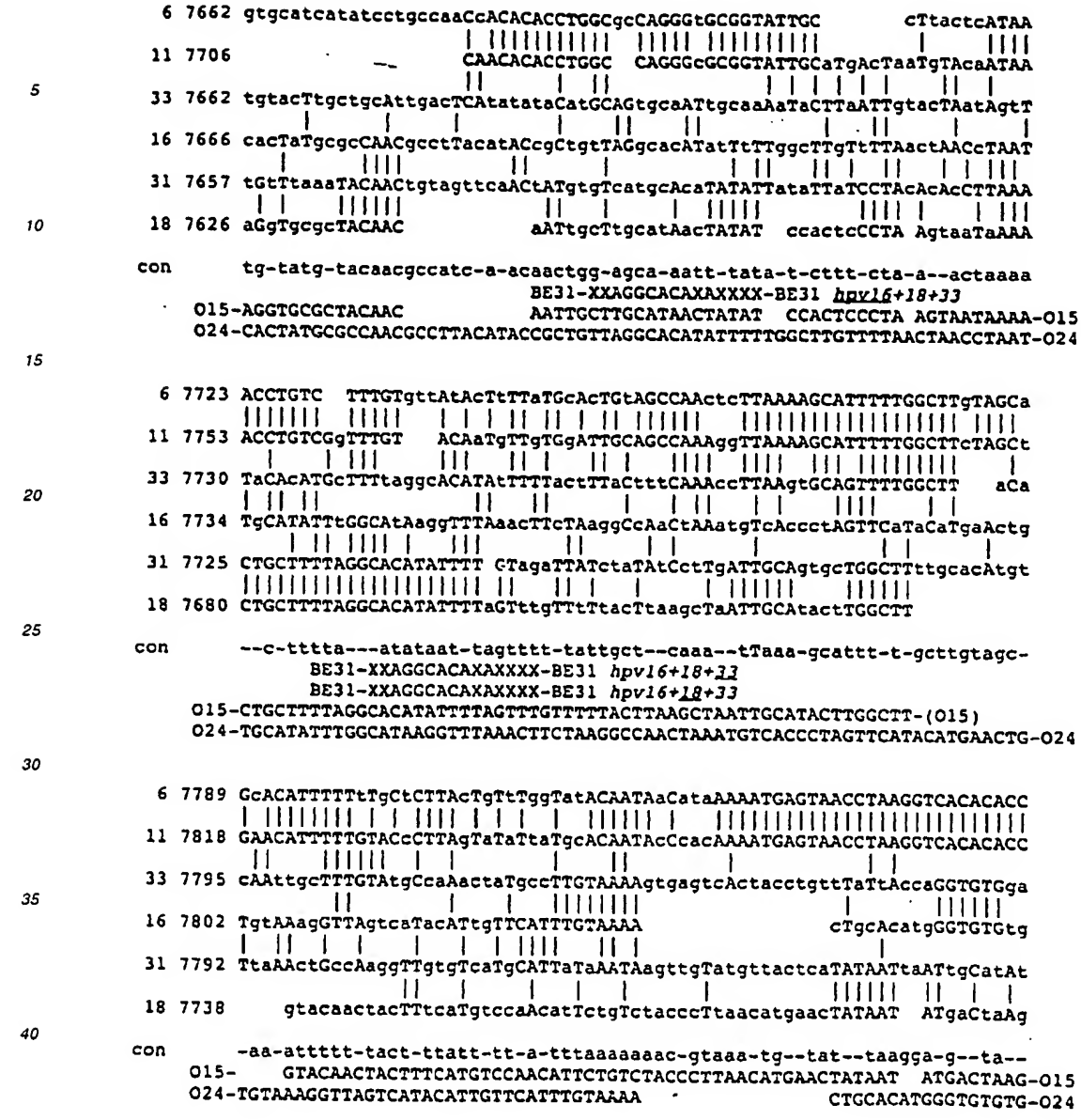
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16 7734 TgCATATTtGGCAtAaggTTTAaacTTcTAaggCcAaCtAAatgTcAccctAGTTCaTaCaTgaActg
31 7725 CTGCTTTTAGGCACATATTTT GTagaTTATctaTAtCctTgATTGCAgtgcTGGCTTttgcacAtgt
18 7680 CTGCTTTTAGGCACATATTTTaGTttgTTtTtacTtaagcTaATTGCAtacttGGCTT
con --c-tttta---atataat-tagtttt-tattgct--caaa--tTaaa-gcattt-t-gcttgtagc-
BE31-XXAGGCACAXAXXXX-BE31 hpv16+18+33
BE31-XXAGGCACAXAXXXX-BE31 hpv16+18+33
O15-CTGCTTTTAGGCACATATTTTAGTTTGTTTTTACTTAAGCTAATTGCATACTTGGCTT-(O15)
O24-TGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTG-O24
6 7789 GcACATTTTTtTgCtCTTAcTgTtTggTatACAATAaCataAAAATGAGTAACCTAAGGTCACACACC
11 7818 GAACATTTTTGTACcCTTAgTaTaTtaTgcACAATAcCcacAAAATGAGTAACCTAAGGTCACACACC
33 7795 cAAttgcTTTGTAtgCcaAactaTgccTTGTAAAAgtgagtcActacctgttTaTtAccaGGTGTGga
16 7802 TgtAAagGTTAgtcaTacATtgTTCATTTGTAAAA cTgcAcatgGGTGTGtg
31 7792 TtaAActGccAaggTTgtgTcaTgCATTaTaAATAagttgTatgttactcaTATAATtaATtgCatAt
18 7738 gtacaactacTTtcaTgtccaAcatTctgTctacccTtaacatgaacTATAAT ATgaCtaAg
con -aa-attttt-tact-ttatt-tt-a-tttaaaaaaac-gtaaa-tg--tat--taagga-g--ta--
O15- GTACAACTACTTTCATGTCCAACATTCTGTCTACCCTTAACATGAACTATAAT ATGACTAAG-O15
O24-TGTAAAGGTTAGTCATACATTGTTCATTTGTAAAA CTGCACATGGGTGTGTG-O24

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  6 7857 TGCgACCGGTTTCGGTTAtCCACACCCTACATATTTCCTTCTTATA
         ||| |||||||||||||| |||||||||||||||||||||||||||
 11 7886 TGCAACCGGTTTCGGTTAcCCACACCCTACATATTTCCTTCTTATA
           | |   ||| ||| |       | |  |    || ||  ||||
 33 7863 CtAACCG TTTTaGGTcAtaTTggtCATTTA tAaTCtTTTATATAATA
         | ||||| ||||       ||   ||||||  |     ||||||||||
 16 7854 CaAACCGATTTT     gGGTTACACATTTACAAGCAAcTTATATAATAATACTaa
              |          |||||||  |||||||||||  |  | |  | |||||
 31 7860 agGTattAcaccgtTTTcGGTTACAGtTTTACAAGCAAtTGtTCTTtTTATACT
           ||           |||  |  || |   ||        |  | |  ||||||
 18 7800 ctGTgcatacatagTTTatGcaACcGaaaTAggttgggcaGcaCaTacTATACTtttc

con      cg-aac---ttt-ggttatg--acccat-tA-a-ttc-tt-ttataataatact----
     015-CTGTGCATACATAGTTTATGCAACCGAAATAGGTTGGGCAGCACATACTATACTTTTC-(015)
     024-CAAACCGATTTT     GGGTTACACATTTACAAGCAACTTATATAATAATACTAA(-024)
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Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, NL, SE

1. A composition useful in LCR for amplifying the DNA of human papilloma virus present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the following oligonucleotide sets:

LCR5:	SEQ ID No.	
	81	GCTGCAAACA ACTATACATG ATATAA,
	82	TTATATCATG TATAGTTGTT TGCAGC,
	83	TATTAGAATG TGTGTACTGC AAGCA,
	84	TGCTTGCAGT ACACACATTC TAATA;
LCR6:	SEQ ID No.	
	85	CTTCACTGCA AGACATAGAA ATAA,
	86	TTATTTCTAT GTCTTGCAGT GAA,
	87	CCTGTGTATA TTGCAAGACA GTAT,
	88	TACTGTCTTG CAATATACAC AGG;
LCR7:	SEQ ID No.	
	89	TATATTGCAA GACAGTATTG GAAC,
	90	GTTCCAATAC TGTCTTGCAA TTTA,
	91	TTACAGAGGT ATTTGAATTT GCATT,
	92	AATGCAAATT CAAATACCTC TGTAA; and
LCR8:	SEQ ID No.	
	93	GTATGGAACA ACATTAGAAC AGCA,
	94	TGCTGTTCTA ATGTTGTTCC ATAC,
	95	ATACAACAAA CCGTTGTGTG ATTT,
	96	AAATCACACA ACGGTTTGTT GTAT.

2. A composition according to claim 1 for amplifying the DNA of human papilloma virus type 1 6 present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the following oligonucleotide sets:
LCR5 (SEQ ID Nos. 81,82,83 and 84) and LCR8 (SEQ ID Nos. 93, 94, 95 and 96).

3. A composition according to claim 1 for amplifying the DNA of human papilloma virus type 18 present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the following oligonucleotide sets:
LCR 6(SEQ ID Nos. 85,86,87 and 88) and LCR 7 (SEQ ID Nos. 89,90,91 and 92).

4. A kit for detecting the presence of human papilloma virus DNA in a test sample, comprising:
a composition according to any of claims 1 to 3; and
further comprising a ligase.

5. A kit according to claim 4, wherein said ligase is thermostable.

6. A composition useful in PCR for amplifying the DNA of human papilloma virus present in a test sample, said composition comprising:

a first nucleic acid primer of sense direction, capable of hybridizing to the antisense strand of HPV DNA , said primer having from 10 to about 30 nucleotides in length and having a sequence selected from the group consisting of the following sequences:

SEQ ID No.	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
6	GAATTAGTTA GACCATTTAA AAG,
7	GGGGAAACAC CAGAATGGAT A,
81	GCTGCAAACA ACTATACATG ATATAA,
85	CTTCACTGCA AGACATAGAA ATAA,
89	TATATTGCAA GACAGTATTG GAAC and
93	GTATGGAACA ACATTAGAAC AGCA; and

a second nucleic acid primer of antisense direction, capable of hybridizing to the sense strand of HPV DNA, said primer having from 10 to about 30 nucleotides in length and having a sequence selected from the group consisting of the following sequences:

SEQ ID No.	
5	AGGTGTCAGG AAAACCAAAT TTATT,
84	TGCTTGCAGT ACACACATTC TAATA,
88	TACTGTCTTG CAATATACAC AGG,
92	AATGCAAATT CAAATACCTC TGTAA and
96	AAATCACACA ACGGTTTGTT GTAT;

provided said first and second primers hybridize to their respective antisense and sense strands at locations such that their 3' ends do not overlap and, in the direction of extension, the 5' ends of said primers are spaced further apart than the 3' ends of said primers.

7. A composition according to claim 6 wherein said first and second primers are selected from the following pairs of oligonucleotide sequences (identified by Sequence ID No.):
1 and 5, 6 and 5, 7 and 5, 81 and 84,
85 and 88, 89 and 92, and 93 and 96.

8. A kit for detecting the presence of human papilloma virus DNA in a test sample, comprising:
a composition according to claim 6 or 7 ; and
further comprising a polymerase.

9. A kit according to claim 8 wherein said polymerase is thermostable.

10. A consensus oligonucleotide for hybridizing human papilloma virus types 6, 11, 16, 18, 31, 33 and 61, which oligonucleotide comprises from about 10 to about 60 nucleotides in length and is selected from the group of sequences consisting of:

SEQ ID No.	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
5	AGGTGTCAGG AAAACCAAAT TTATT,
6	GAATTAGTTA GACCATTTAA AAG and
7	GGGGAAACAC CAGAATGGAT A;

and their complements.

11. A type-specific oligonucleotide for determining the presence of human papilloma virus type 16, having a sequence selected from the group consisting of:

SEQ ID No.	
81	GCTGCAAACA ACTATACATG ATATAA,
82	TTATATCATG TATAGTTGTT TGCAGC,
83	TATTAGAATG TGTGTACTGC AAGCA,
84	TGCTTGCAGT ACACACATTC TAATA,
93	GTATGGAACA ACATTAGAAC AGCA,
94	TGCTGTTCTA ATGTTGTTCC ATAC,
95	ATACAACAAA CCGTTGTGTG ATTT and
96	AAATCACACA ACGGTTTGTT GTAT;

and their complements.

12. A type-specific oligonucleotide for determining the presence of human papilloma virus type 18, having a sequence selected from the group consisting of: SEQ ID No.

SEQ ID No.	
85	CTTCACTGCA AGACATAGAA ATAA,
86	TTATTTCTAT GTCTTGCAGT GAA,
87	CCTGTGTATA TTGCAAGACA GTAT,
88	TACTGTCTTG CAATATACAC AGG,
89	TATATTGCAA GACAGTATTG GAAC,
90	GTTCCAATAC TGTCTTGCAA TTTA,
91	TTACAGAGGT ATTTGAATTT GCATT and
92	AATGCAAATT CAAATACCTC TGTAA;

and their complements.

13. A method for determining the presence of any human papilloma virus in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one consensus oligonucleotide selected from the group of claim 10, said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

14. A method for determining the presence of human papilloma virus type 16 in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one oligonucleotide selected from the group of claim 11, said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

15. A method for determining the presence of human papilloma virus type 18 in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one oligonucleotide selected from the group of claim 12, said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

16. A method according to any of claims 13-15, further comprising a step of amplification prior to or concurrent with said hybridizing step.

17. A method according to claim 16, wherein said amplification step comprises PCR or LCR.

Claims for the following Contracting States : ES

1. A composition useful in LCR for amplifying the DNA of human papilloma virus present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the following oligonucleotide sets:

LCR5: SEQ ID No.

81	GCTGCAAACA ACTATACATG ATATAA,
82	TTATATCATG TATAGTTGTT TGCAGC,
83	TATTAGAATG TGTGTACTGC AAGCA,
84	TGCTTGCAGT ACACACATTC TAATA;

LCR6: SEQ ID No.

85	CTTCACTGCA AGACATAGAA ATAA,
86	TTATTTCTAT GTCTTGCAGT GAA,
87	CCTGTGTATA TTGCAAGACA GTAT,
88	TACTGTCTTG CAATATACAC AGG;

LCR7: SEQ ID No.

89	TATATTGCAA GACAGTATTG GAAC,
90	GTTCCAATAC TGTCTTGCAA TTTA,
91	TTACAGAGGT ATTTGAATTT GCATT,
92	AATGCAAATT CAAATACCTC TGTAA; and

LCR8: SEQ ID No.

93	GTATGGAACA ACATTAGAAC AGCA,
94	TGCTGTTCTA ATGTTGTTCC ATAC,
95	ATACAACAAA CCGTTGTGTG ATTT,
96	AAATCACACA ACGGTTTGTT GTAT.

2. A composition according to claim 1 for amplifying the DNA of human papilloma virus type 16 present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the fol lowing oligonucleotide sets :
LCR5 (SEQ ID Nos. 81,82,83 and 84) and LCR8 (SEQ ID Nos. 93, 94, 95 and 96).

3. A composition according to claim 1 for amplifying the DNA of human papilloma virus type 18 present in a test sample, said composition comprising a set of four oligonucleotide probes, said probe sets being selected from the group consisting of the following oligonucleotide sets:
LCR6(SEQ ID Nos. 85,86,87 and 88) and LCR 7(SEQ ID Nos. 89,90,91 and 92).

4. A kit for detecting the presence of human papilloma virus DNA in a test sample, comprising:

a composition according to any of claims 1 to 3; and further comprising a ligase.

5. A kit according to claim 4, wherein said ligase is thermostable.

6. A composition useful in PCR for amplifying the DNA of human papilloma virus present in a test sample, said composition comprising:

a first nucleic acid primer of sense direction, capable of hybridizing to the antisense strand of HPV DNA, said primer having from 10 to about 30 nucleotides in length and having a sequence selected from the group consisting of the following sequences:

SEQ ID No.	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
6	GAATTAGTTA GACCATTTAA AAG,
7	GGGGAAACAC CAGAATGGAT A,
81	GCTGCAAACA ACTATACATG ATATAA,
85	CTTCACTGCA AGACATAGAA ATAA,
89	TATATTGCAA GACAGTATTG GAAC and
93	GTATGGAACA ACATTAGAAC AGCA; and

a second nucleic acid primer of antisense direction, capable of hybridizing to the sense strand of HPV DNA, said primer having from 10 to about 30 nucleotides in length and having a sequence selected from the group consisting of the following sequences:

SEQ ID No.	
5	AGGTGTCAGG AAAACCAAAT TTATT,
84	TGCTTGCAGT ACACACATTC TAATA,
88	TACTGTCTTG CAATATACAC AGG,
92	AATGCAAATT CAAATACCTC TGTAA and
96	AAATCACACA ACGGTTTGTT GTAT;

provided said first and second primers hybridize to their respective antisense and sense strands at locations such that their 3' ends do not overlap and, in the direction of extension, the 5' ends of said primers are spaced further apart than the 3' ends of said primers.

7. A composition according to claim 6 wherein said first and second primers are selected from the following pairs of oligonucleotide sequences (identified by Sequence ID No.):
1 and 5, 6 and 5, 7 and 5, 81 and 84,
85 and 88, 89 and 92, and 93 and 96.

8. A kit for detecting the presence of human papilloma virus DNA in a test sample, comprising:
a composition according to claim 6 or 7; and
further comprising a polymerase.

9. A kit according to claim 8 wherein said polymerase is thermostable.

10. A method for determining the presence of any human papilloma virus in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one consensus oligonucleotide selected from the group of sequences consisting of:

SEQ ID No.	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
5	AGGTGTCAGG AAAACCAAAT TTATT,
6	GAATTAGTTA GACCATTTAA AAG and
7	GGGGAAACAC CAGAATGGAT A;

and their complements,
said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

11. . A method for determining the presence of human papilloma virus type 16 in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one oligonucleotide selected from the group of sequences consisting of:

SEQ ID No.	
81	GCTGCAAACA ACTATACATG ATATAA,
82	TTATATCATG TATAGTTGTT TGCAGC,
83	TATTAGAATG TGTGTACTGC AAGCA,
84	TGCTTGCAGT ACACACATTC TAATA,
93	GTATGGAACA ACATTAGAAC AGCA,
94	TGCTGTTCTA ATGTTGTTCC ATAC,
95	ATACAACAAA CCGTTGTGTG ATTT and
96	AAATCACACA ACGGTTTGTT GTAT;

and their complements, said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

12. . A method for determining the presence of human papilloma virus type 18 in a test sample, comprising:

a. hybridizing DNA in the test sample with at least one oligonucleotide selected from the group of sequences consisting of:

SEQ ID No.	
85	CTTCACTGCA AGACATAGAA ATAA,
86	TTATTTCTAT GTCTTGCAGT GAA,
87	CCTGTGTATA TTGCAAGACA GTAT,
88	TACTGTCTTG CAATATACAC AGG,
89	TATATTGCAA GACAGTATTG GAAC,
90	GTTCCAATAC TGTCTTGCAA TTTA,
91	TTACAGAGGT ATTTGAATTT GCATT and
92	AATGCAAATT CAAATACCTC TGTAA;.

and their complements,
said oligonucleotide being conjugated to a signal generating compound capable of producing a detectable signal; and
b. determining the presence of human papilloma virus by detecting the signal generated.

13. A method according to any of claims 10-12 , further comprising a step of amplification prior to or concurrent with said hybridizing step.

14. A method according to claim 13, wherein said amplification step comprises PCR or LCR.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, NL, SE

1. Zusammensetzung, die für die LCR ("ligase chain reaction", Ligasekettenreaktion) zur Vervielfachung der DNA des humanen Papillomavirus nützlich ist, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht:

LCR5: SEQ ID N r

81 GCTGCAAACA ACTATACATG ATATAA,
82 TTATATCATG TATAGTTGTT TGCAGC,
83 TATTAGAATG TGTGTACTGC AAGCA,
84 TGCTTGCAGT ACACACATTC TAATA;

LCR6: SEQ ID N r

85 CTTCACTGCA AGACATAGAA ATAA,
86 TTATTTCTAT GTCTTGCAGT GAA,
87 CCTGTGTATA TTGCAAGACA GTAT,
88 TACTGTCTTG CAATATACAC AGG;

LCR7: SEQ ID N r

89 TATATTGCAA GACAGTATTG GAAC,
90 GTTCCAATAC TGTCTTGCAA TTTA,
91 TTACAGAGGT ATTTGAATTT GCATT,
92 AATGCAAATT CAAATACCTC TGTAA; und

LCR8: SEQ ID N r

93 GTATGGAACA ACATTAGAAC AGCA,
94 TGCTGTTCTA ATGTTGTTCC ATAC,
95 ATACAACAAA CCGTTGTGTG ATTT,
96 AAATCACACA ACGGTTTGTT GTAT.

2. Zusammensetzung nach Anspruch 1 zur Vervielfachung der DNA des humanen Papillomavirus Typ 16, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht: LCR5 (SEQ ID Nrn 81, 82, 83 und 84) und LCR8 (SEQ ID Nm 93, 94, 95 und 96)

3. Zusammensetzung nach Anspruch 1 zur Vervielfachung der DNA des humanen Papillomavirus Typ 18, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht: LCR6 (SEQ ID Nrn 85, 86, 87 und 88) und LCR7 (SEQ ID Nm 89, 90, 91 und 92).

4. Kit zum Nachweis der Anwesenheit der DNA des humanen Papillomavirus in einer Testprobe, das folgendes umfaßt:
eine Zusammensetzung nach einem der Ansprüche 1 bis 3, und des weiteren eine Ligase.

5. Kit nach Anspruch 4, worin die Ligase thermostabil ist.

6. Zusammensetzung, die bei der PCR ("polymerase chain reaction" Polymerasekettenreaktion) zur Vervielfachung der DNA des humanen Papillomavirus nützlich ist, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung folgendes umfaßt:

einen ersten Nukleinsäureprimer, der zur Richtung gleichläufig ist, welcher zur Hybridisierung an den gegenläufigen Strang der HPV-DNA befähigt ist, wobei der Primer 10 bis ungefähr 30 Nukleotide lang ist und eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus den folgenden Sequenzen besteht:

1	CAGATGTCTC TGTGGCGGCC TAGTG,	
6	GAATTAGTTA GACCATTTAA AAG,	
7	GGGGAAACAC CAGAATGGAT A,	
81	GCTGCAAACA ACTATACATG ATATAA,	
85	CTTCACTGCA AGACATAGAA ATAA,	
89	TATATTGCAA GACAGTATTG GAAC	und
93	GTATGGAACA ACATTAGAAC AGCA;	und

einen zweiten Nukleinsäureprimer, der zur Richtung gegenläufig ist, welcher zur Hybridisierung an den gleichläufigen Strang der HPV-DNA befähigt ist, wobei der Primer 10 bis ungefähr 30 Nukleotide lang ist und eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus den folgenden Sequenzen besteht:

SEQ ID Nr

5	AGGTGTCAGG AAAACCAAAT TTATT,	
84	TGCTTGCAGT ACACACATTC TAATA,	
88	TACTGTCTTG CAATATACAC AGG,	
92	AATGCAAATT CAAATACCTC TGTAA	und
96	AAATCACACA ACGGTTTGTT GTAT;	

vorausgesetzt, daß der erste und der zweite Primer an ihre jeweiligen gleich- und gegenläufigen Stränge an solchen Stellen hybridisieren, daß ihre 3'-Enden nicht überlappen, und daß die 5'-Enden der Primer in Verlängerungsrichtung weiter räumlich abgesetzt sind als die 3'-Enden der Primer.

7. Zusammensetzung nach Anspruch 6, worin der erste und zweite Primer aus den folgenden Paaren von Oligonukleotidsequenzen (die durch die Sequenz ID Nr bezeichnet sind) gewählt sind:
1 und 5, 6 und 5, 7 und 5, 81 und 84,
85 und 88, 89 und 92, und 93 und 96.

8. Kit zum Nachweis der Anwesenheit der DNA des humanen Papillomavirus in einer Testprobe, das folgendes umfaßt:
eine Zusammensetzung nach Anspruch 6 oder ,7 und des weiteren eine Polymerase.

9. Kit nach Anspruch 8, worin die Polymerase thermostabil ist.

10. Consensus-Oligonukleotid zur Hybridisierung der humanen papillomaviren Typ 6, 11, 16, 18, 31, 33 und 61, wobei das Oligonukleotid ungefähr 10 bis ungefähr 60 Oligonukleotide lang ist und aus der Gruppe von Sequenzen gewählt ist, die aus folgendem besteht:

SEQ ID Nr

1	CAGATGTCTC TGTGGCGGCC TAGTG,	
5	AGGTGTCAGG AAAACCAAAT TTATT,	
6	GAATTAGTTA GACCATTTAA AAG	
7	GGGGAAACAC CAGAATGGAT A;	und

und aus deren Komplementen.

11. Typ-spezifisches Oligonukleotid zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 16, das eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus folgendem besteht:

SEQ ID Nr

81	GCTGCAAACA ACTATACATG ATATAA,	
82	TTATATCATG TATAGTTGTT TGCAGC,	
83	TATTAGAATG TGTGTACTGC AAGCA,	
84	TGCTTGCAGT ACACACATTC TAATA,	
93	GTATGGAACA ACATTAGAAC AGCA,	
94	TGCTGTTCTA ATGTTGTTCC ATAC,	
95	ATACAACAAA CCGTTGTGTG ATTT	und
96	AAATCACACA ACGGTTTGTT GTAT;	

und aus deren Komplementen.

12. Typ-spezifisches Oligonukleotid zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 18, das eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus folgendem besteht:

SEQ ID Nr

85	CTTCACTGCA AGACATAGAA ATAA,	
86	TTATTTCTAT GTCTTGCAGT GAA,	
87	CCTGTGTATA TTGCAAGACA GTAT,	
88	TACTGTCTTG CAATATACAC AGG,	
89	TATATTGCAA GACAGTATTG GAAC,	
90	GTTCCAATAC TGTCTTGCAA TTTA,	
91	TTACAGAGGT ATTTGAATTT GCATT	und
92	AATGCAAATT CAAATACCTC TGTAA;	

und aus deren Komplementen.

13. Verfahren zur Bestimmung der Anwesenheit irgendeines humanen Papillomavirus in einer Testprobe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Consensus-Oligonukleotid, das aus der Gruppe nach Anspruch 10 gewählt ist, wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und
b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

14. Verfahren zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 16 in einer Probe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Oligonukleotid, das aus der Gruppe nach Anspruch 11 gewählt ist, wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und
b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

15. Verfahren zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 18 in einer Testprobe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Oligonukleotid, das aus der Gruppe nach Anspruch 12 gewählt ist, wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und

b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

16. Verfahren nach einem der Ansprüche 13-15, das des weiteren einen Vervielfachungsschritt umfaßt, der vor oder in Konkurrenz mit dem Hybridisierungsschritt stattfindet.

17. Verfahren nach Anspruch 16, worin der Vervielfachungsschritt PCR oder LCR umfaßt.

Patentansprüche für folgenden Vertragsstaat : ES

1. Zusammensetzung, die für die LCR ("ligase chain reaction", Ligasekettenreaktion) zur Vervielfachung der DNA des humanen Papillomavirus nützlich ist, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht:

LCR5: SEQ ID Nr

81 GCTGCAAACA ACTATACATG ATATAA,
82 TTATATCATG TATAGTTGTT TGCAGC,
83 TATTAGAATG TGTGTACTGC AAGCA,
84 TGCTTGCAGT ACACACATTC TAATA;

LCR6: SEQ ID Nr

85 CTTCACTGCA AGACATAGAA ATAA,
86 TTATTTCTAT GTCTTGCAGT GAA,
87 CCTGTGTATA TTGCAAGACA GTAT,
88 TACTGTCTTG CAATATACAC AGG;

LCR7: SEQ ID Nr

89 TATATTGCAA GACAGTATTG GAAC,
90 GTTCCAATAC TGTCTTGCAA TTTA,
91 TTACAGAGGT ATTTGAATTT GCATT,
92 AATGCAAATT CAAATACCTC TGTAA;

LCR8: SEQ ID Nr

und

93 GTATGGAACA ACATTAGAAC AGCA,
94 TGCTGTTCTA ATGTTGTTCC ATAC,
95 ATACAACAAA CCGTTGTGTG ATTT,
96 AAATCACACA ACGGTTTGTT GTAT.

2. Zusammensetzung nach Anspruch 1 zur Vervielfachung der DNA des humanen Papillomavirus Typ 16, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht: LCR5 (SEQ ID Nrn 81, 82, 83 und 84) und LCR8 (SEQ ID Nrn 93, 94, 95 und 96).

3. Zusammensetzung nach Anspruch 1 zur Vervielfachung der DNA des humanen Papillomavirus TYP 18, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung einen Satz von vier Oligonukleotidsonden umfaßt, wobei die Sondensätze aus der Gruppe gewählt sind, die aus den folgenden Oligonukleotidsätzen besteht: LCR6 (SEQ ID Nrn 85, 86, 87 und 88) und LCR7 (SEQ ID Nrn 89, 90, 91 und 92).

4. Kit zum Nachweis der Anwesenheit der DNA des humanen Papillomavirus in einer Testprobe, das folgendes umfaßt:
eine Zusammensetzung nach einem der Ansprüche 1 bis 3, und des weiteren eine Ligase.

5. Kit nach Anspruch 4, worin die Ligase thermostabil ist.

6. Zusammensetzung, die bei der PCR ("polymerase chain reaction" polymerasekettenreaktion) zur Vervielfachung der DNA des humanen Papillomavirus nützlich ist, der in einer Testprobe vorhanden ist, wobei die Zusammensetzung folgendes umfaßt:

einen ersten Nukleinsäureprimer, der zur Richtung gleichläufig ist, welcher zur Hybridisierung an den gegenläufigen Strang der HPV-DNA befähigt ist, wobei der Primer 10 bis ungefähr 30 Nukleotide lang ist und eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus den folgenden Sequenzen besteht:

SEQ ID Nr		
1	CAGATGTCTC TGTGGCGGCC TAGTG,	
6	GAATTAGTTA GACCATTTAA AAG,	
7	GGGGAAACAC CAGAATGGAT A,	
81	GCTGCAAACA ACTATACATG ATATA,	
85	CTTCACTGCA AGACATAGAA ATAA,	
89	TATATTGCAA GACAGTATTG GAAC	und
93	GTATGGAACA ACATTAGAAC AGCA;	und

einen zweiten Nukleinsäureprimer, der zur Richtung gegenläufig ist, welcher zur Hybridisierung an den gleichläufigen Strang der HPV-DNA befähigt ist, wobei der Primer 10 bis ungefähr 30 Nukleotide lang ist und eine Sequenz aufweist, die aus der Gruppe gewählt ist, die aus den folgenden Sequenzen besteht:

SEQ ID Nr		
5	AGGTGTCAGG AAAACCAAAT TTATT,	
84	TGCTTGCAGT ACACACATTC TAATA,	
88	TACTGTCTTG CAATATACAC AGG,	
92	AATGCAAATT CAAATACCTC TGTAA	und
96	AAATCACACA ACGGTTTGTT GTAT;	und

vorausgesetzt, daß der erste und der zweite Primer an ihre jeweiligen gleich- und gegenläufigen Stränge an solchen Stellen hybridisieren, daß ihre 3'-Enden nicht überlappen, und daß die 5'-Enden der Primer in Verlängerungsrichtung weiter räumlich abgesetzt sind als die 3'-Enden der Primer.

7. Zusammensetzung nach Anspruch 6, worin der erste und zweite Primer aus den folgenden Paaren von Oligonukleotidsequenzen (die durch die Sequenz ID Nr bezeichnet sind) gewählt sind:
1 und 5, 6 und 5, 7 und 5, 81 und 84,
85 und 88, 89 und 92, und 93 und 96.

8. Kit zum Nachweis der Anwesenheit der DNA des humanen Papillomavirus in einer Testprobe, das folgendes umfaßt:
eine Zusammensetzung nach Anspruch 6 oder 7, und des weiteren eine Polymerase.

9. Kit nach Anspruch 8, worin die Polymerase thermostabil ist.

10. Verfahren zur Bestimmung der Anwesenheit irgendeines humanen papillomavirus in einer Testprobe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Consensus-Oligonukleotid, das aus der Gruppe von Sequenzen gewählt ist, die aus folgendem besteht:

SEQ ID Nr

1	CAGATGTCTC TGTGGCGGCC TAGTG,
5	AGGTGTCAGG AAAACCAAAT TTATT
6	GAATTAGTTA GACCATTTAA AAG und
7	GGGGAAACAC CAGAATGGAT A;

und aus deren Komplementen,
wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und
b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

11. Verfahren zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 16 in einer Probe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Oligonukleotid, das aus der Gruppe von Sequenzen gewählt ist, die aus folgendem besteht:

SEQ ID Nr

81	GCTGCAAACA ACTATACATG ATATAA,
82	TTATATCATG TATAGTTGTT TGCAGC,
83	TATTAGAATG TGTGTACTGC AAGCA,
84	TGCTTGCAGT ACACACATTC TAATA,
93	GTATGGAACA ACATTAGAAC AGCA,
94	TGCTGTTCTA ATGTTGTTCC ATAC,
95	ATACAACAAA CCGTTGTGTG ATTT und
96	AAATCACACA ACGGTTTGTT GTAT;

und aus deren Komplementen,
wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und
b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

12. Verfahren zur Bestimmung der Anwesenheit des humanen Papillomavirus Typ 18 in einer Testprobe, das folgendes umfaßt:

a. Hybridisieren der DNA in der Testprobe mit wenigstens einem Oligonukleotid, das aus der Gruppe von Sequenzen gewählt ist, die aus folgendem besteht:

SEQ ID Nr	
85	CTTCACTGCA AGACATAGAA ATAA,
86	TTATTTCTAT GTCTTGCAGT GAA,
87	CCTGTGTATA TTGCAAGACA GTAT,
88	TACTGTCTTG CAATATACAC AGG,
89	TATATTGCAA GACAGTATTG GAAC,
90	GTTCCAATAC TGTCTTGCAA TTTA,
91	TTACAGAGGT ATTTGAATTT GCATT und
92	AATGCAAATT CAAATACCTC TGTAA;

und aus deren Komplementen,
wobei das Oligonukleotid an eine signalerzeugende Verbindung konjugiert ist, die zur Erzeugung eines nachweisbaren Signals befähigt ist, und
b. Bestimmen der Anwesenheit des humanen Papillomavirus, indem das erzeugte Signal nachgewiesen wird.

13. Verfahren nach einem der Ansprüche 10-12, das des weiteren einen Vervielfachungsschritt umfaßt, der vor oder in Konkurrenz mit dem Hybridisierungsschritt stattfindet.

14. Verfahren nach Anspruch 13, worin der vervielfachungsschritt PCR oder LCR umfaßt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, NL, SE

1. Composition utile dans la LCR pour amplifier l'ADN de virus du papillome humain présent dans échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :

LCR5 :	n° d'identification	
	81	GCTGCAAACA ACTATACATG ATATAA,
	82	TTATATCATG TATAGTTGTT TGCAGC,
	83	TATTAGAATG TGTGTACTGC AAGCA,
	84	TGCTTGCAGT ACACACATTC TAATA ;

LCR6 :	n° d'identification	
	85	CTTCACTGCA AGACATAGAA ATAA,
	86	TTATTTCTAT GTCTTGCAGT GAA,
	87	CCTGTGTATA TTGCAAGACA GTAT,
	88	TACTGTCTTG CAATATACAC AGG ;

LCR7 :	n° d'identification	
	89	TATATTGCAA GACAGTATTG GAAC
	90	GTTCCAATAC TGTCTTGCAA TTTA,
	91	TTACAGAGGT ATTTGAATTT GCATT,
	92	AATGCAAATT CAAATACCTC TGTAA ; et

LCR8 :	n° d'identification	
	93	GTATGGAACA ACATTAGAAC AGCA,
	94	TGCTGTTCTA ATGTTGTTCC ATAC,
	95	ATACAACAAA CCGTTGTGTG ATTT,
	96	AAATCACACA ACGGTTTGTT GTAT.

2. Composition selon la revendication 1, destinée à amplifier l'ADN de virus du papillome humain de type 16 présent dans un échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :
LCR5 (n° d'identification 81, 82, 83 et 84) et LCR8 (n° d'identification 93, 94, 95 et 96).

3. Composition selon la revendication 1, destinée à amplifier l'ADN de virus du papillome humain de type 18 présent dans un échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :
LCR6 (n° d'identification 85, 86, 87 et 88) et LCR7 (n° d'identification 89, 90, 91 et 92).

4. Kit de détection de la présence d'ADN de virus du papillome humain dans un échantillon à doser, comprenant : une composition selon l'une quelconque des revendications 1 à 3, et en outre une ligase.

5. Kit selon la revendication 4, dans lequel ladite ligase est thermostable.

6. Composition utile dans la PCR pour amplifier l'ADN de virus du papillome humain présent dans un échantillon à doser, ladite composition comprenant :

une première amorce d'acide nucléique de direction sens, capable de s'hybrider au brin antisens de l'ADN de HPV, ladite amorce ayant de 10 à environ 30 nucléotides de long et une séquence sélectionnée dans le groupe constitué par les séquences suivantes :

N° d'identification	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
6	GAATTAGTTA GACCATTTAA AAG,
7	GGGGAAACAC CAGAATGGAT A,
81	GCTGCAAACA ACTATACATG ATATAA,
85	CTTCACTGCA AGACATAGAA ATAA,
89	TATATTGCAA GACAGTATTG GAAC et
93	GTATGGAACA ACATTAGAAC AGCA ; et

une deuxième amorce d'acide nucléique de direction antisens, capable de s'hybrider au brin sens de l'ADN

de HPV, ladite amorce ayant de 10 à environ 30 nucléotides de long et une séquence sélectionnée dans le groupe constitué par les séquences suivantes :

N° d'identification	
5	AGGTGTCAGG AAAACCAAAT TTATT,
84	TGCTTGCAGT ACACACATTC TAATA,
88	TACTGTCTTG CAATATACAC AGG,
92	AATGCAAATT CAAATACCTC TGTAA et
96	AAATCACACA ACGGTTTGTT GTAT ;

pour autant que lesdites première et deuxième amorces s'hybrident à leurs brins respectifs antisens et sens à des emplacements tels que leurs extrémités 3' ne se chevauchent pas et que, dans la direction d'extension, les extrémités 5' desdites amorces soient plus espacées que les extrémités 3' desdites amorces.

7. Composition selon la revendication 6, dans laquelle lesdites première et deuxième amorces sont sélectionnées parmi les paires suivantes de séquences oligonucléotidiques (identifiées par leur numéro d'identification) :
1 et 5, 6 et 5, 7 et 5, 81 et 84,
85 et 88, 89 et 92, et 93 et 96.

8. Kit de détection de la présence d'ADN de virus du papillome humain dans un échantillon à doser, comprenant :
une composition selon la revendication 6 ou 7 et en outre une polymérase.

9. Kit selon la revendication 8, dans lequel ladite polymérase est thermostable.

10. Oligonucléotide consensus pour hybridation du virus du papillome humain des types 6, 11, 16, 18, 31, 33 et 61, lequel oligonucléotide a d'environ 10 à environ 60 nucléotides de long et est sélectionné dans le groupe de séquences constitué par :

N° d'identification	
1	CAGATGTCTC TGTGGCGGCC TAGTG,
5	AGGTGTCAGG AAAACCAAAT TTATT,
6	GAATTAGTTA GACCATTTAA AAG et
7	GGGGAAACAC CAGAATGGAT A ;

et leurs compléments.

11. Oligonucléotide spécifique d'un type, destiné à déterminer la présence du virus du papillome humain de type 16, ayant une séquence sélectionnée dans le groupe constitué par :

N° d'identification	
81	GCTGCAAACA ACTATACATG ATATAA,
82	TTATATCATG TATAGTTGTT TGCAGC,
83	TATTAGAATG TGTGTACTGC AAGCA,
84	TGCTTGCAGT ACACACATTC TAATA,
93	GTATGGAACA ACATTAGAAC AGCA,
94	TGCTGTTCTA ATGTTGTTCC ATAC,
95	ATACAACAAA CCGTTGTGTG ATTT et
96	AAATCACACA ACGGTTTGTT GTAT ;

et leurs compléments.

12. Oligonucléotide spécifique d'un type, destiné à déterminer la présence du virus du papillome humain de type 18, ayant une séquence sélectionnée dans le groupe constitué par :

N° d'identification			
85	CTTCACTGCA	AGACATAGAA	ATAA,
86	TTATTTCTAT	GTCTTGCAGT	GAA,
87	CCTGTGTATA	TTGCAAGACA	GTAT,
88	TACTGTCTTG	CAATATACAC	AGG,
89	TATATTGCAA	GACAGTATTG	GAAC,
90	GTTCCAATAC	TGTCTTGCAA	TTTA,
91	TTACAGAGGT	ATTTGAATTT	GCATT et
92	AATGCAAATT	CAAATACCTC	TGTAA ;

et leurs compléments.

13. Procédé de détermination de la présence d'un virus quelconque du papillome humain dans un échantillon à doser, comprenant :

    a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide consensus sélectionné dans le groupe selon la revendication 10, ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
    b. la détermination de la présence du virus du papillome humain par détection du signal émis.

14. Procédé de détermination de la présence du virus du papillome humain de type 16 dans un échantillon à doser, comprenant :

    a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide sélectionné dans le groupe selon la revendication 11, ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
    b. la détermination de la présence du virus du papillome humain par détection du signal émis.

15. Procédé de détermination de la présence du virus du papillome humain de type 18 dans un échantillon à doser, comprenant :

    a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide sélectionné dans le groupe selon la revendication 12, ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
    b. la détermination de la présence du virus du papillome humain par détection du signal émis.

16. Procédé selon une quelconque des revendications 13 à 15, comprenant en outre une étape d'amplification avant ou pendant ladite étape d'hybridation.

17. Procédé selon la revendication 16, dans lequel ladite étape d'amplification comprend la PCR ou la LCR.

Revendications pour l'Etat contractant suivant : ES

1. Composition utile dans la LCR pour amplifier l'ADN de virus du papillome humain présent dans échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :

LCR5 : n° d'identification			
81	GCTGCAAACA	ACTATACATG	ATATAA,
82	TTATATCATG	TATAGTTGTT	TGCAGC,
83	TATTAGAATG	TGTGTACTGC	AAGCA,
84	TGCTTGCAGT	ACACACATTC	TAATA ;

LCR6 :	n° d'identification			
	85	CTTCACTGCA	AGACATAGAA	ATAA,
	86	TTATTTCTAT	GTCTTGCAGT	GAA,
	87	CCTGTGTATA	TTGCAAGACA	GTAT,
	88	TACTGTCTTG	CAATATACAC	AGG ;

LCR7 :	n° d'identification			
	89	TATATTGCAA	GACAGTATTG	GAAC
	90	GTTCCAATAC	TGTCTTGCAA	TTTA,
	91	TTACAGAGGT	ATTTGAATTT	GCATT,
	92	AATGCAAATT	CAAATACCTC	TGTAA ; et

LCR8 :	n° d'identification			
	93	GTATGGAACA	ACATTAGAAC	AGCA,
	94	TGCTGTTCTA	ATGTTGTTCC	ATAC,
	95	ATACAACAAA	CCGTTGTGTG	ATTT,
	96	AAATCACACA	ACGGTTTGTT	GTAT.

2. Composition selon la revendication 1, destinée à amplifier l'ADN de virus du papillome humain de type 16 présent dans un échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :
LCR5 (n° d'identification 81, 82, 83 et 84) et LCR8 (n° d'identification 93, 94, 95 et 96).

3. Composition selon la revendication 1, destinée à amplifier l'ADN de virus du papillome humain de type 18 présent dans un échantillon à doser, ladite composition comprenant un ensemble de quatre sondes oligonucléotidiques, lesdits ensembles de sondes étant sélectionnés dans le groupe constitué par les ensembles d'oligonucléotides suivants :
LCR6 (n° d'identification 85, 86, 87 et 88) et LCR7 (n° d'identification 89, 90, 91 et 92).

4. Kit de détection de la présence d'ADN de virus du papillome humain dans un échantillon à doser, comprenant :
une composition selon l'une quelconque des revendications 1 à 3, et en outre une ligase.

5. Kit selon la revendication 4, dans lequel ladite ligase est thermostable.

6. Composition utile dans la PCR pour amplifier l'ADN de virus du papillome humain présent dans un échantillon à doser, ladite composition comprenant :

une première amorce d'acide nucléique de direction sens, capable de s'hybrider au brin antisens de l'ADN de HPV, ladite amorce ayant de 10 à environ 30 nucléotides de long et une séquence sélectionnée dans le groupe constitué par les séquences suivantes :

N° d'identification			
1	CAGATGTCTC	TGTGGCGGCC	TAGTG,
6	GAATTAGTTA	GACCATTTAA	AAG,
7	GGGGAAACAC	CAGAATGGAT	A,
81	GCTGCAAACA	ACTATACATG	ATATAA,
85	CTTCACTGCA	AGACATAGAA	ATAA,
89	TATATTGCAA	GACAGTATTG	GAAC et
93	GTATGGAACA	ACATTAGAAC	AGCA ; et

une deuxième amorce d'acide nucléique de direction antisens, capable de s'hybrider au brin sens de l'ADN de HPV, ladite amorce ayant de 10 à environ 30 nucléotides de long et une séquence sélectionnée dans le groupe constitué par les séquences suivantes :

N° d'identification			
5	AGGTGTCAGG	AAAACCAAAT	TTATT,
84	TGCTTGCAGT	ACACACATTC	TAATA,
88	TACTGTCTTG	CAATATACAC	AGG,
92	AATGCAAATT	CAAATACCTC	TGTAA et
96	AAATCACACA	ACGGTTTGTT	GTAT ;

pour autant que lesdites première et deuxième amorces s'hybrident à leurs brins respectifs antisens et sens à des emplacements tels que leurs extrémités 3' ne se chevauchent pas et que, dans la direction d'extension, les extrémités 5' desdites amorces soient plus espacées que les extrémités 3' desdites amorces.

7. Composition selon la revendication 6, dans laquelle lesdites première et deuxième amorces sont sélectionnées parmi les paires suivantes de séquences oligonucléotidiques (identifiées par leur numéro d'identification) :
1 et 5, 6 et 5, 7 et 5, 81 et 84,
85 et 88, 89 et 92, et 93 et 96.

8. Kit de détection de la présence d'ADN de virus du papillome humain dans un échantillon à doser, comprenant :
une composition selon la revendication 6 ou 7 et
en outre une polymérase.

9. Kit selon la revendication 8, dans lequel ladite polymérase est thermostable.

10. Procédé de détermination de la présence d'un virus quelconque du papillome humain dans un échantillon à doser, comprenant :

a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide consensus sélectionné dans le groupe de séquences constitué par :

N° d'identification			
1	CAGATGTCTC	TGTGGCGGCC	TAGTG,
5	AGGTGTCAGG	AAAACCAAAT	TTATT,
6	GAATTAGTTA	GACCATTTAA	AAG et
7	GGGGAAACAC	CAGAATGGAT	A ;

et leurs compléments, ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
b. la détermination de la présence du virus du papillome humain par détection du signal émis.

11. Procédé de détermination de la présence du virus du papillome humain de type 16 dans un échantillon à doser, comprenant :

a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide sélectionné dans le groupe de séquences constitué par :

N° d'identification			
81	GCTGCAAACA	ACTATACATG	ATATAA,
82	TTATATCATG	TATAGTTGTT	TGCAGC,
83	TATTAGAATG	TGTGTACTGC	AAGCA,
84	TGCTTGCAGT	ACACACATTC	TAATA,
93	GTATGGAACA	ACATTAGAAC	AGCA,
94	TGCTGTTCTA	ATGTTGTTCC	ATAC,
95	ATACAACAAA	CCGTTGTGTG	ATTT et
96	AAATCACACA	ACGGTTTGTT	GTAT ;

et leurs compléments,
ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
b. la détermination de la présence du virus du papillome humain par détection du signal émis.

12. Procédé de détermination de la présence du virus du papillome humain de type 18 dans un échantillon à doser, comprenant :

a. l'hybridation de l'ADN dans l'échantillon à doser avec au moins un oligonucléotide sélectionné dans le groupe de séquences constitué par :

N° d'identification			
85	CTTCACTGCA	AGACATAGAA	ATAA,
86	TTATTTCTAT	GTCTTGCAGT	GAA,
87	CCTGTGTATA	TTGCAAGACA	GTAT,
88	TACTGTCTTG	CAATATACAC	AGG,
89	TATATTGCAA	GACAGTATTG	GAAC,
90	GTTCCAATAC	TGTCTTGCAA	TTTA,
91	TTACAGAGGT	ATTTGAATTT	GCATT et
92	AATGCAAATT	CAAATACCTC	TGTAA ;

et leurs compléments,
ledit oligonucléotide étant conjugué à un composé émetteur d'un signal, capable de produire un signal détectable, et
b. la détermination de la présence du virus du papillome humain par détection du signal émis.

13. Procédé selon une quelconque des revendications 10 à 12, comprenant en outre une étape d'amplification avant ou pendant ladite étape d'hybridation.

14. Procédé selon la revendication 13, dans lequel ladite étape d'amplification comprend la PCR ou la LCR.

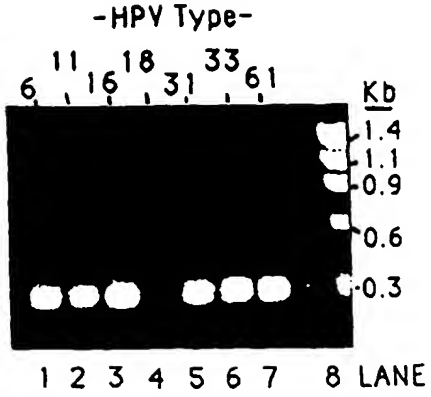


FIG. 1

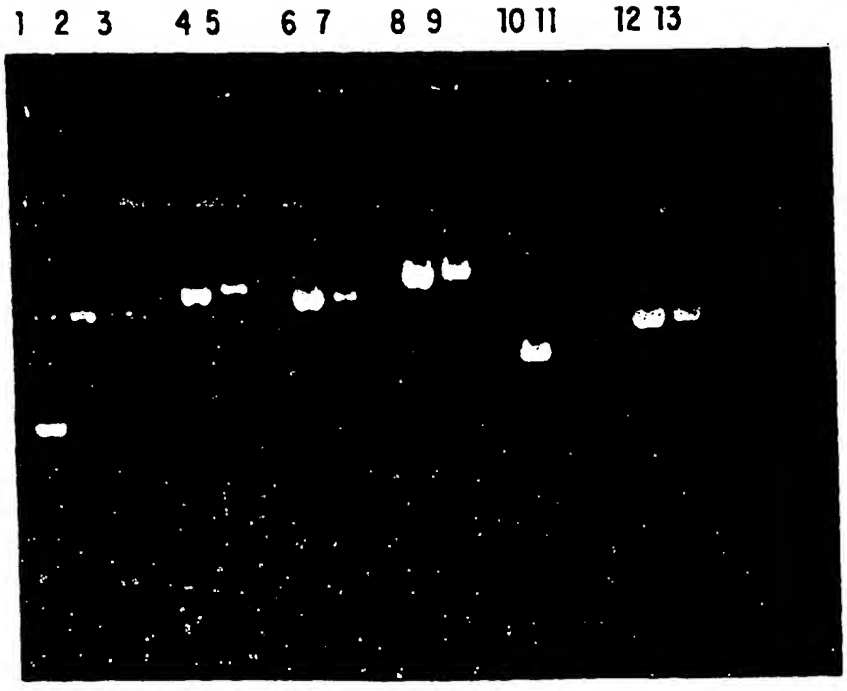


FIG. 2

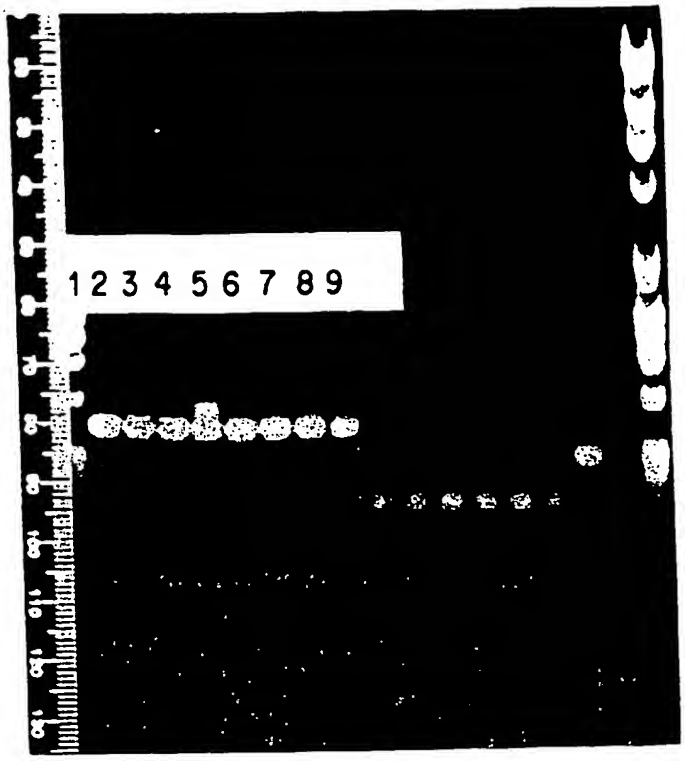


FIG. 3

FIG. 4

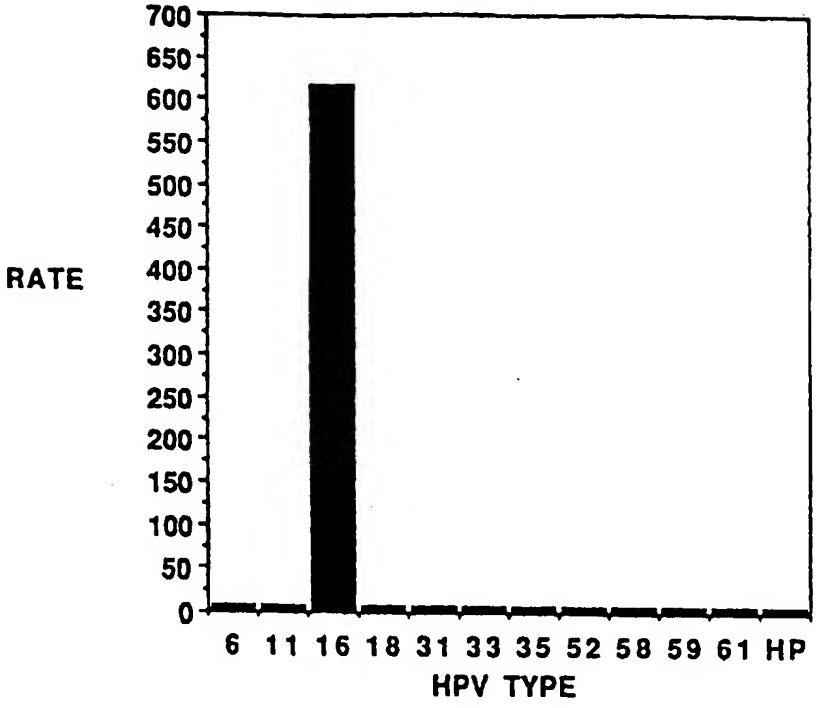

700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
RATE
6 11 16 18 31 33 35 52 58 59 61 HP
HPV TYPE

FIG. 5

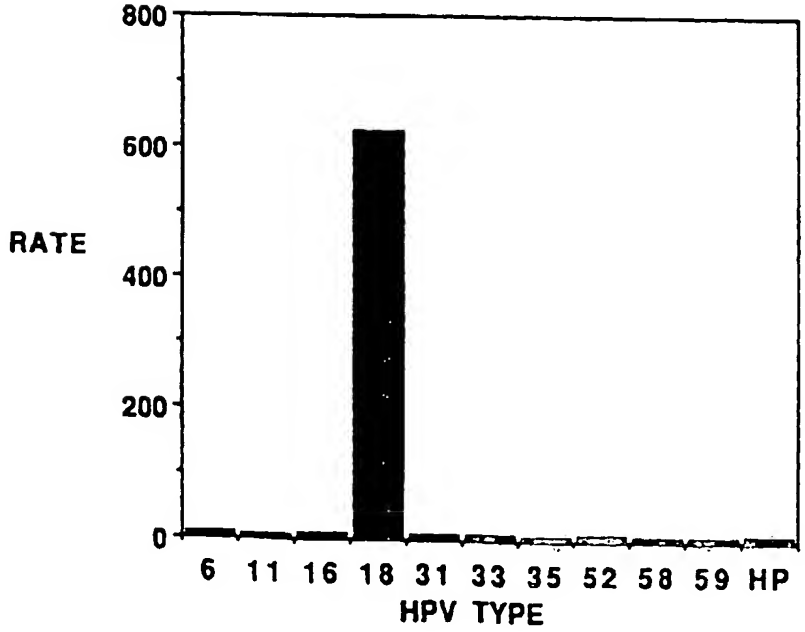

800
600
400
200
0
RATE
6 11 16 18 31 33 35 52 58 59 HP
HPV TYPE

FIG. 6

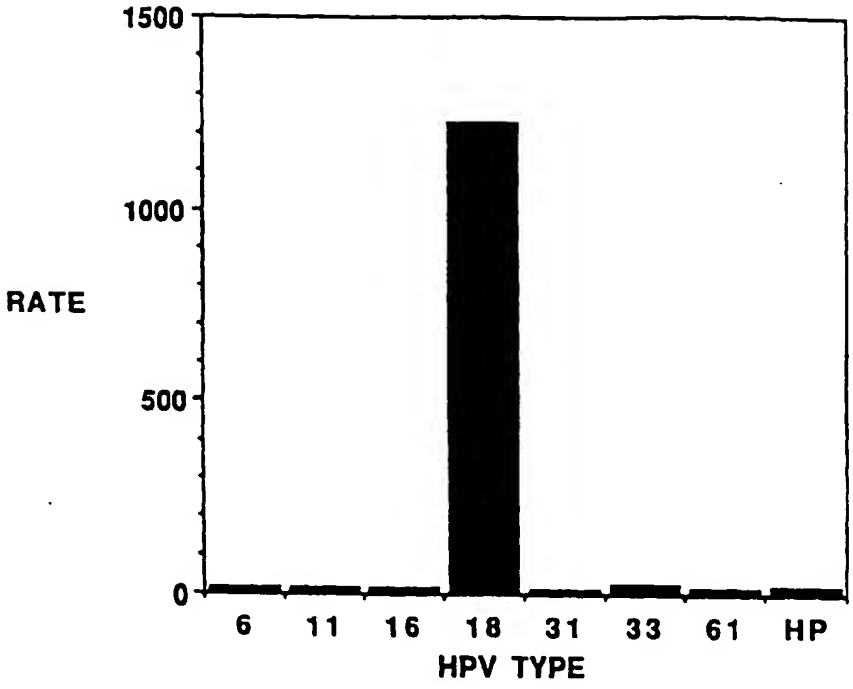
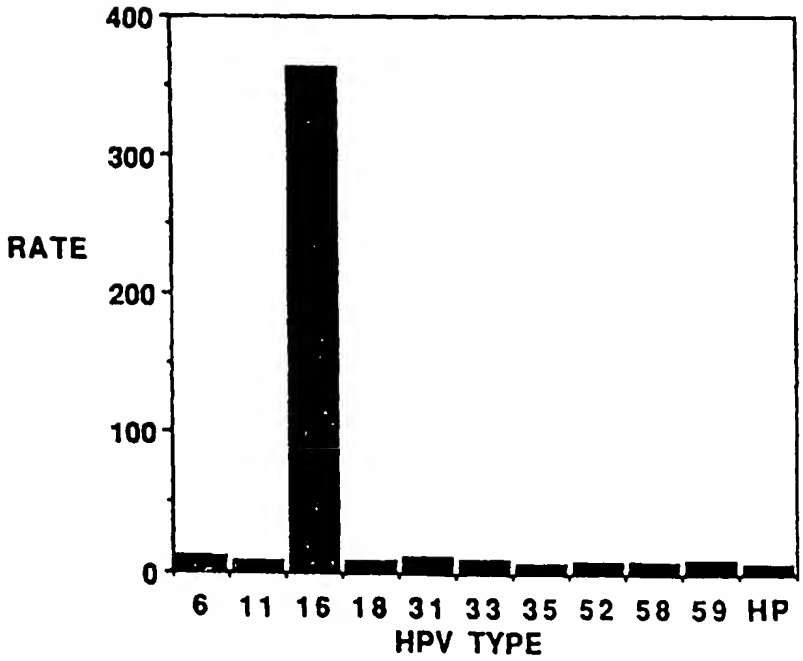

1500
1000
500
0
RATE
6
11
16
18
31
33
61
HP
HPV TYPE

FIG. 7


400
300
200
100
0
RATE
6
11
16
18
31
33
35
52
58
59
HP
HPV TYPE